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Stefano Ratti[1], Sara Mongiorgi[1], Giulia Ramazzotti[1], Matilde Y. Follo[1], Giulia A. Mariani[1], Pann-Ghill Suh[2], James A. McCubrey[3], Lucio Cocco[1*] and Lucia Manzoli[1]

[1] Cellular Signalling Laboratory, Department of Biomedical and Neuromotor Sciences, University of Bologna, via Irnerio 48, 40126 Bologna, Italy

[2] School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, Republic of Korea

[3] Department of Microbiology & Immunology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, USA

[*] Correspondence to: Lucio Cocco, Cellular Signalling Laboratory, Department of Biomedical and Neuromotor Sciences, University of Bologna, via Irnerio 48, 40126 Bologna, Italy. E-mail: lucio.cocco@unibo.it

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ABSTRACT: the existence of an independent nuclear inositide pathway distinct from the cytoplasmic one has been demonstrated in different physiological systems and in diseases. In this prospect we analyze the role of PI-PLCβ1 nuclear isoform in relation to the cell cycle regulation, the cell differentiation and different physiopathological pathways focusing on the importance of the nuclear localization from both molecular and clinical point of view. PI-PLCβ1 is essential for G1/S transition through DAG and Cyclin D3 and plays also a central role in G2/M progression through Cyclin B1 and PKCα. In the differentiation process of C2C12 cells PI-PLCβ1 increases in both myogenic differentiation and osteogenic differentiation. PI-PLCβ1 and Cyclin D3 reduction has been observed in Myotonic Dystrophy (DM) suggesting a pivotal role of these enzymes in DM physiopathology. PI-PLCβ1 is also involved in adipogenesis through a double phase mechanism. Moreover PI-PLCβ1 plays a key role in the normal hematopoietic differentiation where it seems to decrease in erythroid differentiation and increase in myeloid differentiation. In Myelodysplastic Syndromes (MDS) PI-PLCβ1 has a genetic and epigenetic relevance and it is related to MDS patients' risk of Acute Myeloid Leukemia (AML) evolution. In MDS patients PI-PLCβ1 seems to be also a therapeutic predictive outcome marker. In the central nervous system PI-PLCβ1 seems to be involved in different pathways in both brain cortex development and synaptic plasticity related to different diseases. Another PI-PLC isozyme that could be related to nuclear activities is PI-PLCζ that is involved in infertility processes.

KEY WORDS: SIGNALING, INOSITIDES, NUCLEUS, PHOSPHOLIPASE C, PATHOPHYSIOLOGY

NUCLEAR INOSITIDE SIGNALING

During the last 30 years nuclear inositide signaling has been investigated in many different systems and diseases. The eukaryotic outer nuclear membrane is made of protein and phospholipids and comes from the endoplasmic reticulum. For this reason cell fractionation showed nuclei with a high amount of lipids, due to the incomplete removal of the nuclear double layer. After the total removal of the nuclear envelope by detergents it was interesting to observe that still a significant quantity of lipids was present inside the nucleus. Even though nuclear Phosphatidylcholine (PC) is the main phospholipid, Phosphatidylinositols (PIs), present in small amount (Tribble et al., 2016), have specific role in intranuclear signaling therefore in nuclear function regulation and behave differently from their counterparts in the cytoplasm. The autonomous nuclear PI cycle in eukaryotic cells is involved in different regulation processes, from cell proliferation to differentiation and many others (Irvine, 2003). In this prospect we will try to describe the state of the art of nuclear inositide signaling via Phospolipase C (PLC), the pathophysiological involvement of PI-PLCs, the fields remained undiscovered and the possible researches related to this intriguing and complicated world.

HISTORICAL BACKGROUND

In 1987, for the first time, using Friend cell highly purified nuclei (washed free of nuclear membrane by Triton) Cocco et al. suggested that Phosphatydilinostil-4-phosphate (PI4P) and Phosphatydilinostil-4,5-biphosphate (PI(4,5)P_2) were specifically present inside the nucleus. This work proposed that these PIs could have an important role in signal transduction parallel to the more documented cytoplasmic one (Cocco et al., 1987). Later, the stimulation with Insulin-like growth factor-1 (IGF-1) of Swiss 3T3 fibroblasts demonstrated the IGF-1 regulation of a distinct nuclear PI metabolism (Cocco et al., 1988). Moreover the treatment of Swiss 3T3 fibroblasts with IGF-1 determined a decrease in nuclear PI4P and PI(4,5)P_2 with a consequential increase in Diacylglycerol (DAG) and therefore a translocation of protein kinase C (PKC) inside the nucleus. On the contrary Swiss 3T3 fibroblast treated with bombesin showed activation only of the classical cytoplasmic pathway (Divecha et al.,1991, Martelli et al., 1991). The presence of these enzymes at the nuclear level suggests their essential role in nuclear signaling that, otherwise, would not be possible to be realized at the cytoplasmic level only. Moreover for being able to identify a certain amount of these PIs inside the nucleus their presence should be stable or, at least, long enough to be localized and of course to perform a certain nuclear activity. For these reasons these data opened a new field of research in nuclear inositide signaling via PLC, independent from the cytoplasmic one. Since the beginning of these experiments up to now there have been lots of enlightenments on this theme, but many aspects remain still unclear and critical.

NUCLEAR INOSITIDE SUBCELLULAR LOCALIZATION AND FUNCTIONS

If it is known that PIs are also present within the nucleus, their precise topography is still under investigation. Different studies focused on nuclear compartments in order to clarify PI nuclear geography. The main difficulty is that some PIs have different possible routes of synthesis and also removal that are localized in different regions inside and outside the nucleus (Irvine, 2003). For example PI(4,5)P_2 could be synthetized by PIP-5-kinase (PIP5K) or by PIP-4-kinase (PIP4K) starting from different substrates, respectively PI-4-phosphate(PI-4P) and PI-5P (Bulley et al., 2015). PIP5K and PIP4K have both different isoforms with different localization from the plasma membrane to

the nucleus itself: PIP5Kα (plasma membrane), PIP5Kβ (both plasma membrane and perinuclear region), and PIP5Kγ (in focal adhesions and adherent junctions in epithelial cells); PIP4K2A (plasma membrane and cytoplasm), PIP4K2B (nucleus) and PIP4K2C (endomembrane compartment) (Bultsma, et al. 2010). Considering our geographical focus the main question here is which class of these two PIPKs is responsible for the $PIP(4,5)P_2$ synthesis inside the nucleus. Several experiments evidenced that PIP5K was accountable of almost 90% of $PI(4,5)P_2$ production within the nucleus, while PIP4K was responsible for only a little amount (Balla & Balla, 2006). These data suggest that there are several possible pathways for the same PI synthesis, related to its localization and function. Moreover PI4-kinase is mainly present in nuclear peripheral matrix, instead PI-PLC, DAG kinase, and PI4P5-kinase seem to be present in the internal one (Payrastre et al., 1992). On rat liver cells the isolation of nuclei with Triton X-100 at a concentration of 0,04%, in order to remove completely the bilayer nuclear envelope, showed that there were still several nuclear pools of different PIs such as PI, $PI(4,5)P_2$, phosphatidic acid (PA), DAG, and phosphatidylinositol 3,4,5-triphosphate ($PI(3,4,5)P_3$) (Vann et al., 1997). Moreover it has been evidenced that $PI(4,5)P_2$ is related to nuclear speckles. These speckles are interchromatin granule clusters where different PIs and other related enzymes are localized regulating chromatin remodeling, nuclear functions and RNA splicing (Boronenkov et al., 1998, Osborne et al., 2001).

The complexity of this topic is related to the difficulties of reproducible nuclear purification techniques and the variables in the metabolism and localization of these enzymes. In any case, these data suggest that PIs are somehow and at some point present inside specific nuclear regions and can therefore perform nuclear functions. Their temporary or stable permanence within the nucleus should then be useful for some nuclear activities that, instead, would not be possible in the cytoplasm (Tribble et al., 2016).

Indeed nuclear PIs are involved in many activities and can control several fundamental nuclear processes. As we saw above, their localization in specific nuclear regions named speckles strongly suggests their role in transcription regulation and in chromatin remodeling (Boronenkov et al., 1998). Proteins can bind PIs through Plant Homeo Domains (PHD) fingers making the interaction with other nuclear proteins, ligands, histones and chromatin possible. PIs can modify histone N-tails characteristics regulating the activity of several enzymes involved in chromatin remodeling, leading to different chromatin architecture and therefore gene transcription. Moreover they are able to modulate gene transcription interacting with transcription complexes and nuclear proteins. PIs can also directly affect RNA maturation affecting several gene expression and therefore PIs can have pivotal roles in different physiopathological processes (Shah et al., 2013). Between all the PIs that are present in the nucleus of eukaryotic cells we will mainly focus on the PI-PLCs and its related enzymes trying to analyze their physiopathological role.

NUCLEAR PI-PLC FEATURES

PLCs are a class of 13 isozymes, divided in six families: PI-PLC-β (1-4), -γ (1-2), -δ (1, 3 and 4), -ε, -ζ and η (1-2)(Gresset et al., 2012). These different enzymes share conserved structural features and can all hydrolyze $PI(4,5)P_2$ in response to several ligand bonds with cell surface receptors. This process produces DAG and inositol-1,4,5-trisphosphate (IP_3). IP_3 is responsible for the release of Ca2+ that, together with DAG, leads to the activation of PKC. This molecular cascade is essential for transmembrane signal transductions that result in cellular communication, proliferation and differentiation. Beyond these routes, PLCs are also localized within the nucleus and are independent from the cytoplasmic pathways. As seen before, we now know that several PIs are

localized inside the nucleus itself, but the specific mechanism of activation is still uncertain. Cytoplasmic PLC activation is related to G-protein-coupled receptor (GPCR) and receptor tyrosine kinase (RTK), but the nuclear activation apparatus is not completely defined. It is possible that IGF-I dependent signal, through a negative feedback by PKC α, modulates nuclear PI-PLC activation (Xu, et al.,2001). Therefore it seems that the nuclear route has different initial triggers and different mechanism compared to the cytoplasmic ones. Between the different PLC isozymes we will focus on PI-PLCβ1, the most investigated PI-PLC within the nucleus (Martelli et al., 1992).
PI-PLCβ1 gene is located on the short arm of chromosome 20 (20p12) and produces two splicing variants: PI-PLCβ1a (150 kDa) and PI-PLCβ1b (140 kDa) that differentiate one another for the C-terminal sequence and their localization (Bahk et al., 1998). Specifically, both of them have a Nuclear Localization Sequence (NLS), but PLCβ1a also has a Nuclear Export Sequence (NES), and for this reason it localizes also in the cytoplasm (Follo et al., 2006). On the other hand, PI-PLCβ1b is mainly located inside the nucleus (Martelli et al., 2005). In order to clarify the mechanism of PI-PLCβ1 nuclear import, PLCb1a isoform was mutated at the C-terminal sequence. This experiment resulted in a significant reduction in the nuclear localization of the enzyme confirming that the C-terminal sequence is involved in the nuclear import (Kim, et al.,1996).

NUCLEAR INOSITIDE SIGNALLING IN CELL CYCLE REGULATION

We will now analyze the role of this enzyme in cell cycle regulation even if we have to consider the difficulties of synchronizing or arresting in a specific cell cycle phase our cell population. Moreover, working with lipids makes always hard to obtain highly purified nuclei in order to be sure to observe the nuclear PI polls.

***Nuclear PI-PLCβ1*:** Nuclear PI-PLCβ1 is involved in cell cycle progression and can regulate the levels of different cyclines such as cyclin D3, cyclin E and cyclin B1. Cells overexpressing PI-PLCβ1 show an increase of Cyclin D3 levels and a higher percentage of cells in S phase, compared to the controls in K562 human erythroleukemia cell line (Poli et al., 2016a). Moreover PLCβ1 can regulate Cyclin E that plays a pivotal role in S-phase progression (Piazzi et al., 2015). PI-PLCβ1 can therefore modulate G1/S cell cycle transition through these cyclines. Another class of nuclear PIs that very recently resulted to be essential during this cell cycle phase in K562 cells are the Diacylglycerol Kinases (DGKs) and particularly the DGKα (Poli et al., 2016b). Furthermore it is interesting to observe that PI-PLCβ1 can be also involved in G2/M cell cycle progression. In 2005 Lukinovic-Skudar et al. described two picks of PLC during both G1/S transition and G2/M progression (Lukinovic-Skudar et al., 2005). We will now analyze the nuclear PI-PLCβ1 mainly related PIs.

***Nuclear DAG*:** as it occurs in the cytoplasm, also in the nucleus PI-PLC activity is responsible for DAG and IP3 synthesis. IP3 should perform his activity as a Ca^{2+} mobilizer inside the nucleus, too and nuclear DAG shows level changes during the cell cycle. As before, it seems that DAG follows nuclear PI-PLC oscillations observed in G1/S and G2/M phases (Lukinovic-Skudar et al., 2005). These data gave more strength to the previous experiments performed on HL60 cell lines (Sun et al.,1997) and U937 cell lines (Deacon et al., 2002) underlining the pivotal role of PLCβ1 and its related enzymes in cell cycle regulation. It has been demonstrated a peak in nuclear DAG production followed by an increase of PKCα and cyclin B1 in G2/M K562 synchronized cells. Indeed the inhibition of PLC activity leads to a reduction in nuclear IP_3 synthesis with a consequential decrease in nuclear translocation of PKCα and Cyclin B1 that are essential in G2/M phase (Poli et

al., 2014). As previously shown the role of PI-PLC is central for the PI cascade in cell cycle regulation and interaction with Cyclins. The probable consequence of DAG generation within the nucleus is an activation of nuclear PKC that leads to several cellular processes.

Nuclear PKC: PKC can be present inside the nucleus in different isoforms (Irvine, 2003)**.** Both its two isoforms PKCα and PKCβII can translocate inside the nucleus after DAG stimulation. As for other PIs, this translocation should be long enough to be observed and to be able to play some role in nuclear pathways. In Swiss 3T3 cell line PKCα can translocate inside the nuclei as a result of a probable nuclear DAG stimuli (Martelli et al., 1991) and also in U937 cell line the translocation of PKCβII inside the nuclei was showed after DAG increase (Deacon et al., 2002). The intriguing data is that PKC can translocate inside the nuclei also after other stimuli such as IGF-1, Platlet derived growth factor or Bryostatin (Irvine, 2003). In addition to these data the same stimuli can determine the translocation in the nucleus of both PKCα and PKCβII isoforms. Another interesting aspect is that a PI-PLCβ1 stable overexpression is related with a decrease of PKCα levels, and this decrease is possibly related to PKCα hyper and stable activation induced by DAG continuous production (Poli et al., 2014). In G2/M-synchronized MEL cells the change in levels of PI-PLCβ1 altered nuclear PKCα accumulation with consecutive alteration in the mitotic process (Fiume et al., 2009). PKC has indeed different targets, like the Lamin B1, strongly linked to the cellular cycle. As we saw above, there is a PI cascade that starting from nuclear PI-PLCβ1, through DAG and IP_3 or and Ca^{2+}, activates PKCα/PKCβII and other molecules controlling the cell cycle. It is possible that DAG and so PKC regulate cell cycle and cell proliferation in relation to different signals and cell population.

***NUCLEAR PI(4,5)P_2*:** PI(4,5)P_2 is not only a substrate for PI-PLC, but this PI seems to be essential in cell cycle progression and other nuclear functions. AS reported before, PI(4,5)P_2 is largely produced by PIP5K that can interact with Retinoblastoma protein (pRb) which, in turn, controls the progression to S phase in the cell cycle (Divecha et al., 2002). The link between PIP5K and pRb can determine PIP5K activation and then PI(4,5)P_2 synthesis. For this reason PI(4,5)P_2 plays an important role in cell cycle progression and in the nuclear PI-PLC cascade. Moreover PI(4,5)P_2 could have structural and also regulatory function in RNA splicing, indeed this class of PIs resides in the interchromatin granular structures interacting dynamically with different chromatin structures during the cell cycle (Osborne et al., 2001) *(Figure 1).*

NUCLEAR PI-PLCβ1 IN CELL DIFFERENTIATION

Nuclear PI-PLCβ1 plays a crucial role in cell differentiation of different cell systems. We will focus on the hematopoietic, osteogenic, myogenic and adipogenic systems in order to concentrate on the different function and variations of this pivotal PI in cell differentiation. Indeed PI-PLCβ1 seems to have reverse modulation in distinctive cell systems *(Figure 2).*

Hematopoietic differentiation: PI-PLCβ1 has shown to be involved in the hematopoietic system performing pivotal roles in both erythroid and myeloid differentiation. Interestingly this nuclear PI has opposite behaviour in these two subsystems: in the erythroid differentiation PI-PLCβ1 seems to decrease, whereas in the myeloid differentiation PI-PLCβ1 appears to increase (Follo et al., 2015). More specifically in murine erythroleukemia cells (MEL) and in normal CD34+ cells with

erythropoietin stimulation towards erythroid lineage PI-PLCβ1 has shown to decrease during the erythroid differentiation process. Furthermore PI-PLCβ1 has presented the same trend also in Myelodysplastic Syndromes (MDS), responding to erythropoiesis stimulating agents (Follo et al., 2012). These data suggest that PI-PLCβ1 plays an inhibiting role in erythroid differentiation that is countered by PI-PLCγ1, another PI-PLC isozyme that increases in erythroid differentiation. The negative role of PI-PLCβ1 in erythroid differentiation was first observed inside the nucleus with an increase of $PI(4,5)P_2$ and a decrease of both PI-PLCβ1 and DAG in differentiating MEL cells (Cocco et al., 1987).

In myeloid differentiation the role of nuclear PI-PLCβ1, studied using a MDS experimental model, seems to be opposite to the one just observed in erythroid differentiation. Indeed MDS cells treated with azacitidine, a hypomethylating agent used for the induction of a normal myeloid differentiation, determined a decrease of PI-PLCβ1 promoter methylation and thus an increase of nuclear PI-PLCβ1. The potential positive role of PI-PLCβ1 in myeloid differentiation could be correlated with the recruitment of specific myeloid transcription factors, such as Myeloid zinc finger-1 (MZF-1), during PI-PLCβ1 expression increase (Cocco et al., 2016). Observing also the clinical aspects of MDS patients treated with azacitidine it is very interesting to point that the increase of PI-PLCβ1 expression is not only related to a favorable clinical response, but also to the durable response to the treatment. Indeed the reduction in PI-PLCβ1 expression can be associated with a loss of response even in patients that had, at first, a positive response to azacitidine (Filì et al., 2013). These data about the variation in PI-PLCβ1 expression could be useful to divide MDS patients that could undergo to azacitidine treatment into two groups: the ones who will probably show a favorable long term response to the drug and the ones who would be refractory to the treatment and thus could avoid its intrinsic risks (Cocco et al., 2015). Afterwards we will see MDS clinical aspects and possibilities related to PI-PLCβ1 and other PIs more in detail.

Osteogenic differentiation: nuclear PI-PLCβ1 seems to be involved also in osteogenic differentiation in relation to different osteogenic molecules. The osteogenic differentiation is stimulated by several signaling pathways, mainly controlled by bone morphogenic proteins (BMPs), Osterix (Osx/Sp7) and runt-related transcription factor 2 (runx2).

C2C12 cells, a mouse skeletal muscle cell line, can differentiate into osteoblasts after BMP-2 stimulation (Katagiri et al., 1994). Very interestingly, during the osteogenesis induced by BMP-2 in C2C12 cells it has been shown an increase of PI-PLCβ1 expression. In addition the down-regulation of PI-PLCβ1 gene expression results in an inhibition of the osteogenic differentiation process induced in C2C12 by BMP-2, as demonstrated by Alkaline phosphatase (ALP) reduction. Instead PI-PLCβ1 overexpression determines an increase of osteogenic differentiation as demonstrated from the rise of ALP and Osx/Sp7 (Ramazzotti et al., 2016). Indeed ALP and Bone gamma-carboxyglutamic acid containing protein (Bglap or Osteocalcin) are osteoblast marker genes that increase in response to the action of the main bone morphogenic proteins that control the osteoblastic differentiation.

PI-PLCβ1 is also related to miR-124, another molecule that is very relevant during the control of osteogenic differentiation. miR-124 can promote the myogenic differentiation and inhibit the osteogenic differentiation by regulating Osx/sp7. Specifically the inhibition of miR-214 in C2C12 cells leads to an increase of PI-PLCβ1 protein level and furthers the osteogenesis (Feng et al., 2011). Another very intriguing aspect of the relation between nuclear PIs and osteogenic differentiation is that, even without BMP-2, the overexpression of PI-PLCβ1 still determines a certain level of osteoblast differentiation, albeit reduced. This could signify the capability of PI-

PLCβ1, per se, to induce osteogenic differentiation through possible downstream molecules.

Myogenic differentiation: There are different pathways related to the myogenic differentiation that can be activated by several events and that can lead to various cellular processes. The two main families of the transcription factors related to myogenic differentiation are the Myogenic Regulatory Factors (MRFs) and the Myocyte Enhancer Facotr-2 (MEF2)(Ramazzotti et al., 2016). C2C12 cells under low serum conditions can differentiate into myotubes. These cells synthesize muscle specific proteins like MyoD and Myogenin (MyoG) that are essential for the myoblast alignment, elongation and fusion that allows myotube formation. During the myotube formation nuclear PI-PLCβ1 increased in C2C12 cells induced to differentiate. This PI-PLCβ1 increase was associated with a Cyclin D3 level increase, too (Cocco et al., 2016). MyoD stimulates Cyclin D3 expression that can thus binds the unphosphorylated pRb responsible for the myoblast cell cycle recession (Ramazzotti et al., 2016). Moreover Cyclin D3 promoter has a binding sequence for PI-PLCβ1, and this bond could be responsible for Cyclin D3 activation in relation to PI-PLCβ1 activity (Faenza et al., 2012). More specifically the transcription factor c-jun needs to bond to Cyclin D3 promoter in order to determine its activation during PI-PLCβ1 signaling cascade (Ramazzotti et al., 2016). This possible pathway starts with PI-PLCβ1 catalytic activity that leads to IP_3 synthesis, which is phosphorylated by Inositol Polyphosphate Multikinase (IPMK) with the production of higher phosphorylated inositol species such as IP4, IP5 and IP6. IP5 can induce β-catenin nuclear translocation, activating c-jun, thus Cyclin D3 promoter. β-catenin target genes are involved in several cellular processes like proliferation, differentiation and cancerogenic stimuli. This pathway could explain the key role of nuclear PI-PLCβ1 in myogenic differentiation. It is worthwhile to remember that also PI-PLCγ1 expression increases during the myogenic differentiation. Interestingly, even if Cyclin D3 is essential in myogenic differentiation it seems not to be involved during osteogenesis. Indeed Cyclin D3 levels do not increase during osteogenesis neither after PI-PLCβ1 overexpression, for this reason it seems to promote myogenic, but not osteogenic differentiation (Ramazzotti et al., 2016). We will analyze later in this prospect the clinical correlation between PI-PLCβ1 and myogenic differentiation in the Myotonic dystrophies.

Adipogenic differentiation: nuclear PI-PLCβ1 and cyclin D3 could be involved in adipocyte differentiation, too. During the differentiation of 3T3-L1 adipocytes there is an up-regulation of the nuclear PI-PLCβ1. Interestingly there have been observed two different phases of this differentiation process. In the first phase, after 5 minutes of incubation with the differentiation media, the pathway is regulated by protein kinase RNA-like endoplasmic reticulum kinase (pERK) and PKCα. In this phase no further nuclear PI-PLCβ1 recruiting is needed, probably because the nuclear PI-PLCβ1 levels are already sufficient at this stage. In the second phase, after 2 days of differentiation, there is the necessity of PI-PLCβ1 nuclear translocation, independently from pERK and PKCα control. Interestingly the overexpression of PLC mutants, lacking the ERK phosphorylation site or the nuclear localization sequence showed that both PI-PLCβ1 phases are essential for an effective differentiation. Moreover the inhibition of PI-PLCβ1 avoided the overexpression of the complex formed by Cyclin D3 and Cyclin-dependent-kinase-4(CDK4), suggesting a role of PI-PLCβ1 in the cell cycle control during adipogenic differentiation (O'Carroll et al., 2009).

NUCLEAR PI-PLCs IN DISEASES

PI-PLCs have been studied as pivotal enzymes, clinical molecular targets and clinical prognostic/diagnostic factors in many physiopathological processes. The different PI-PLC isozymes are related to several pathologies in many human tissues and the understanding of these mechanisms could open new path for developing innovative therapeutic strategies. In this prospect we will focus on the diseases characterized by alterations of nuclear PI-PLCs.

Myelodysplastic Syndromes(MDS): MDS are a heterogeneous group of pathologies characterized by hematopoietic stem cell alteration that lead to anemia, neutropenia, bleeding and infections. Moreover 30% of MDS patients can evolve into acute myeloid leukemia (AML). The IPSS (International Prognostic Scoring Systems) and the WPSS (WHO classification-based Scoring System) classifications, mainly based on the blast number and karyotype, are used to divide MDS patients into two major groups: high and low risk of AML progression. Recently, the evidence of a clinical correlation between the presence of a PI-PLCβ1 monoallelic gene deletion and the progression of MDS to AML opened new perspectives of research and treatments (Follo et al., 2008). The gene encoding for PI-PLCβ1 has been mapped on chromosome 20p12. High-risk MDS patients should undergo allogenic hematopoietic stem cell transplantation (allo-HSCT), but for those who are not candidates, hypomethylating agents (HMAs), such Azacitidine (AZA), are now the first therapeutic choice. HMAs can also be used in low risk MDS patients. AZA is both a hypomethylating and a direct cytotoxic agent for abnormal hematopoietic cells. Patients affected by MDS with a high risk of AML evolution show a reduction in the expression of the nuclear PI-PLCβ1 variant that can also be related to the presence of a mono-allelic deletion of the gene. As PI-PLCβ1b can physiologically regulate the progression through the G1 phase of the cell cycle, the drastic reduction of nuclear PI-PLCβ1 expression could alter the normal cell cycle in patients with MDS. These data could suggest the analysis of PI-PLCβ1 gene expression as a prognostic marker for MDS progression to AML. Nuclear PLCβ1 in MDS is also epigenetically relevant (Jhanwar et al., 2015). In fact, several studies have shown that PLCβ1 is a molecular target for AZA (Follo et al., 2009). As a matter of fact, high-risk and low-risk patients that respond to the drug have shown an early increase of nuclear PI-PLCβ1 expression, a reduction of PI-PLCβ1 promoter methylation, an induction of normal myeloid differentiation and a better prognosis. Moreover, as described above, the increase of PI-PLCβ1 expression and the reduction in PI-PLCβ1 promoter methylation are not only related to a favourable clinical response, but also to a durable response to the treatment. This effect resulted particularly interesting, because, as AZA treatment needed several cycles in order to observe a clinical response, the molecular response could specifically and rapidly predict the future patients' outcome during demethylating therapies. In this way it would be possible to personalize MDS patients' therapies in order to differentiate the ones who would benefit of the treatment from the ones who would be refractory to the treatment and thus could avoid it (Cocco et al., 2016). Furthermore several studies showed that in MDS high-risk patients there is an inverse correlation between PI-PLCβ1 and PI3K expression. PI3K can phosphorylate Protein Kinase B (PKB) also known as Akt (Toker and Marmiroli, 2014). When there is a constitutive activation of Akt, through different phosphorylation, there is a decrease in MDS cell apoptosis that can be reverted after PI-PLCβ1 increase due to the demethylating therapies. Indeed PI-PLCβ1 increase could reduce the level of p-Akt inducing a higher level of apoptosis (Follo et al., 2007). This mechanism is still partially undiscovered, but a possible explication could be that as $PI(4,5)P_2$ is a common substrate of both PI-PLCβ1 and PI3Ks, the alterations in PI-PLCβ1 levels could cause $PI(4,5)P_2$ hydrolysis, reducing its availability for PI3K. This could eventually inhibit Akt activation (Follo et al., 2015). All these mechanisms are extremely interesting for the potentiality of nuclear PI-PLCβ1 to become a prognosis stratification marker and a treatment predictive outcome marker in patients

with MDS.
Nuclear inositides have been studied also in AML that can be a direct evolution of MDS. As we saw earlier in this prospect PIP4K2A catalyzes the phosphorylation of PI5P, synthetizing PI(4,5)P_2. The role of PIP4K2A has been examined in human MLL-AF9 and in murine AML cells, and further investigated also in human AML samples (Jude et al., 2015). AML cells were sensible to PIP4K2A depletion determining a cyclin-dependent kinase inhibitor CDKN1A (p21) accumulation, with thus an activation of the apoptosis. This mechanism seems to be related to the activation of mammalian Target of Rapamycin(mTOR), too. Considering this pathway it is then possible that PIP4K2A could also regulate mTOR. In addition it has been observed significant increase of PI5P following PIP4K2A depletion (Jude et al., 2015). PI5P has been related to TP53 and Akt activation and to cellular reactive oxygen species (ROS) accumulation in normal hematopoietic cells (Jones et al.,2013). Moreover PI5P might increase in response to several stress stimuli, mimicking cell stress induction. This mechanism could induce AML cells to apoptosis or perhaps increase their susceptibility to other critical processes, such as DNA damage. It is still not clear why normal hematopoietic cells and AML cells have different response to PIP4K2A depletion, but these differences could be used for developing new therapeutic strategies against AML (Jude et al., 2015).

Myotonic dystrophies (DM): DM are autosomal dominant neuromuscular degenerative disorders, characterized by a widely variable clinical picture and a slow progressive course, whose onset can occur at any age. The clinical picture is characterized by loss of muscle mass, mytonia, cataracts, defects in the cardiac conduction system, endocrine abnormalities and cognitive deficits in congenital cases. Moreover there is an anticipation phenomenon thus the disease onset tends to occur at an increasingly younger age from generation to generation in the same family.
These diseases are classified into DM type I (DM1) and DM type II (DM2). The majority of patients suffer of DM1, but both DM are caused by DNA tandem repeats that result in aberrant RNA accumulation in the nucleus that causes alteration in RNA-binding protein localization. In DM1 the mutated gene is called myotonic dystrophy protein kinase (DMPK) and encodes a myosin kinase expressed in skeletal muscles. This gene is located in the long arm of chromosome 19 (19q13.3). In the 3'UTR of this locus there is an unstable CTG triplet that can be repeated from 50 to 4000 times when in the normal population the range varies from 5 to 37 times. In DM2 there is a defect in zinc finger protein 9 (ZNF9) gene on chromosome 3 (3q21). The alteration is caused by CCTG expansion from 75 to over 11000, but in this case there seems to be no difference in the severity or the early onset of the disease (Cho and Tapscott, 2007). CUG triplet repeat RNA-binding protein 1 (CUGBP1) plays a central role in alternative splicing of specific target genes and can interact with Initiation Factor 2(eIF2) and Cyclin D3 inducing normal myogenic differentiation. This process can be altered in DM, indeed CUBBP1-eIF2 interactions are reduced in myotonic dystrophy differentiating cells with an impaired muscle differentiation (Faenza et al., 2012). Nevertheless, in DM1, ectopic expression of Cyclin D3 helps the increase of CUBBP1-eIF2 complex, improving the myogenic differentiation marker expression (Salisbury et al., 2008). Moreover, during the myogenesis in DM cells there is a decrease of PI-PLCβ1 expression that could be related to the reduction in Cyclin D3 transcription and induction as described above in the role of PI-PLCβ1 and Cyclin D3 in myogenic differentiation. Interestingly the normalization of PI-PLCβ1 expression in DM1 and DM2 myoblasts can cause a Cyclin D3 expression increase, determining a partially restored phenotype of the myotubes (Faenza et al., 2012). All these data suggest that both PI-PLCβ1 and Cyclin D3 could be investigated for future possible molecular therapies in order to induce a correct skeletal muscle differentiation in DM.

Brain disorders: PI-PLC isozymes are highly expressed in different brain structures. Specific neurotransmitters (NTs) and hormones trigger PI-PLC pathways, determining various activities in many brain regions (Yang et al., 2016). PI-PLCβ1 is present at high concentration in hippocampus, amygdala, lateral septum, olfactory bulb and cerebral cortex (Fukaya et al., 2008). Indeed it has been related to cortical development and synaptic plasticity (Spires et al., 2005). This PI has been associated with epilepsy and to schizophrenia in PI-PLCβ1 knocked out mice (Yang et al., 2016). Losses of PI-PLCβ1 were also observed in patients with epileptic encephalopathies, schizophrenia and bipolar disorders (Kurian et al., 2010,Lo Vasco et al., 2012,Lo Vasco et al., 2013). Also PI-PLCγ1 has been correlated to neuronal cell migration and synaptic plasticity and its activity has been suggest to be of some relevance in epilepsy, Huntington disease, depression, bipolar disorders and Alzheimer disease (Yang et al., 2016). Moreover PI-PLCβ1 seems to be central in the control of endocannabinoid neuronal excitability in most of the brain areas, indeed in the postsynaptic sites, and it's pivotal in the synthesis of DAG which hydrolysis catalyzed by DAG-Lipaseα (DAGLα) leads to 2-arachidonyl-glycerol (2-AG), the most represented CB1 cannabinoid receptor endogenous agonist. Released from the postsynaptic neuron, 2-AG can activate CB1 cannabinoid receptors on the presynaptic sites repressing other NT release (Montaña et al., 2012). Moreover PI-PLCβ1 can respond to two separate signals: depolarization and receptor activation, maintaining the brain inhibitory circuits through 2-AG (del Caño et al., 2014). Interestingly the pathway related to PI-PLCβ1/DAGLα/2-AG seems to be at neuronal nuclear level. In adult rat brain, double immunofluorescence staining and confocal laser scanning showed an overlapping pattern of both PI-PLCβ1 and DAGLα with nuclear speckles marker SC-35 and NeuN/Fox3. In addition PI-PLCβ1 was also highly expressed in neuronal nuclear regions full of $PI(4,5)P_2$. These date were enforced by the fact that the co-localization with Nuclear Pore Complex and Lamin B1 was instead not found (Montaña et al., 2012). There are also evidences of the relation between 2-AG and nuclear Peroxisome proliferator-activated receptor gamma (PPARγ) as a receptor or as a prostaglandin precursor (del Caño et al., 2014). All these intriguing data prompt to better investigate the neuronal nuclear localization and mechanisms of PI-PLCβ1 related molecules in different pathophysiological brain functions. Furthermore, considering nuclear PI-PLCβ1 pivotal role in cell cycle regulation and the controversial and complicate role of cell cycle in several central nervous system insults and pathologies, it would be very fascinating to study nuclear PI-PLCβ1 functions during diverse brain injuries forms (del Caño et al., 2014).

Infertility: PI-PLCζ is a specific sperm protein that has been related to nuclear infertility mechanisms. PI-PLCζ activation and nuclear translocation mechanisms remain unknown, but at nuclear level this PI has been specifically connected with molecular oocyte activation. This PI-PLC is essential in inducing Ca^{2+}release via IP_3 pathway (Saunders et al., 2002). Ca^{2+} oscillations within the oocyte have been related to different processes responsible for its activation such as the cortical granule exocytosis, the release of meiotic arrest, the gene expression regulation, the recruitment of maternal mRNA, the pronuclear formation and the initiating of embryogenesis. The specific mechanism of these reactions is still unclear, but the central role of Ca^{2+} is fundamental for the beginning of embryogenesis (Amdani et al., 2016). The principal cause of infertility after Intra Cytoplasmatic Sperm Injection (ICSI) is considered to be the alteration of oocyte activation mechanisms of which sperm defects seem to be the main responsible (Amdani et al., 2016). From one side, cRNA PI-PLCζ and recombinant protein microinjections in mice and cattle oocyte determine Ca^{2+} oscillations and oocyte activation (Saunders et al., 2002). From the other side

infertile man sperm with alteration in PI-PLCζ quantity and quality fail to induce Ca^{2+} oscillations and therefore oocyte activation (Amdani et al., 2016) . For these reasons it is possible that PI-PLCζ could be pivotal in fertiliy processes and could be considered a prognostic/diagnostic molecular marker in order to identify male patients that could benefit of assisted reproducive technology. Moreover, even if Ca^{2+} ionophores are the main agent for artificial oocyte activation, PI-PLCζ could be a safer potential therapeutic agent (Amdani et al., 2016).

CONCLUSIONS

In this prospect we tried to highlight our understanding of nuclear inositide signaling in different cell systems and pathologies. This topic is at the same time very testing and intriguing due to the technical difficulties in focusing on the nuclear localization of such complicate pathways and to the importance of these enzymes in several pathophysiological mechanisms. We analyzed the PI nuclear signaling through PI-PLCs mainly focusing on PI-PLCβ1, the most studied nuclear PI-PLC, and its related molecules like $PI(4,5)P_2$, DAG, PKCs, cyclin D3 and many others. We started from the beginning of this lipid nuclear study in the late 80s up to the most recent molecular and clinical implications and possibilities. PI-PLCβ1 and its associated molecules have been implicated to be essential in the cell cycle during both G1/S transition and G2/M progression. Moreover PI-PLCβ1 is involved as pivotal enzyme in the normal mesenchymal cell differentiation in osteogenesis, myogenesis and adipogenesis. During the myogenic differentiation of C2C12 cells it has been shown an increase of both nuclear PI-PLCβ1 and Cyclin D3. On the contrary, PI-PLCβ1 and Cyclin D3 reduction has been observed in Myotonic Dystrophy (DM), suggesting a pivotal role of these enzymes in DM physiopathology. Similarly PI-PLCβ1 expression increases in the normal osteogenesis induced by BMP-2 in C2C12 cells and the down-regulation of PI-PLCβ1 gene expression determines an inhibition of the osteogenic differentiation as demonstrated by ALP reduction. PI-PLCβ1 seems to increase also in adipogenesis and its inhibition seems to stop the overexpression of the Cyclin D3-CDK4 complex. This central role has been also demonstrated in the hematopoietic differentiation with probably opposite behaviour between erythroid and myeloid differentiation. Very interestingly the nuclear inositide signaling via PI-PLCβ1 helped to create a bridge between molecular and clinical analyses, giving the opportunity to explore new potential therapeutic strategies and markers. Indeed there have been many progresses especially in understanding MDS pathological mechanism in AML evolution and in response to the therapies. Indeed nuclear PI-PLCβ1 could be used both as a prognostic marker in AML evolution and as a therapy predictive marker in MDS patients. PI-PLCβ1 has been investigated in the central nervous system where it seems to be involved in different processes and pathways in both brain cortex development and synaptic plasticity related to different disease. Nuclear PI-PLCβ1 is also involved in neurotransmitters regulation, suggesting a pivotal role in different brain mechanisms. Another PI-PLC isozyme that could be related to nuclear activities is PI-PLCζ that is involved in oocyte activation and infertility processes where it could be considered as a molecular marker in order to identify male patients that could benefit of assisted reproducive technology. There are certainly many questions that remain to be clarified in relation to nuclear PI cycle and functions, but the importance of these themes and the related clinical prospects stimulate to persist on this field of investigation.

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Ledends to figures:

Figure 1: in this figure we can observe the nuclear PI-PLCβ1 pathway related to the cell cycle. PI-PLCβ1 is essential for G1/S transition through DAG, Cyclin D3-Cdk4 complex and plays a central role in G2/M progression through Cyclin B1 and PKCα. Indeed DAG attracts Cyclin B1 and PKCα from the cytoplasm into the nucleus. We can also see that PIP5K is pivotal for PI(4,5)P2 production within the nucleus, while PIP4K was responsible for only a little amount.

Figure 2: PI-PLCβ1 level changes during cell differentiation. PI-PLCβ1 increases during the normal osteogenic and myogenic differentiation in C2C12 cells. PI-PLCβ1 also increases in adipogenic differentiation in 3T3-L1 cells. During erythroid differentiation there is a decrease in PI-PLCβ1 level demonstrated in MEL, CD34+ and MDS cells. PI-PLCβ1 seems to increase in MDS cells and also in MDS patients samples during the myeloid differentiation induced by Azacitidine.

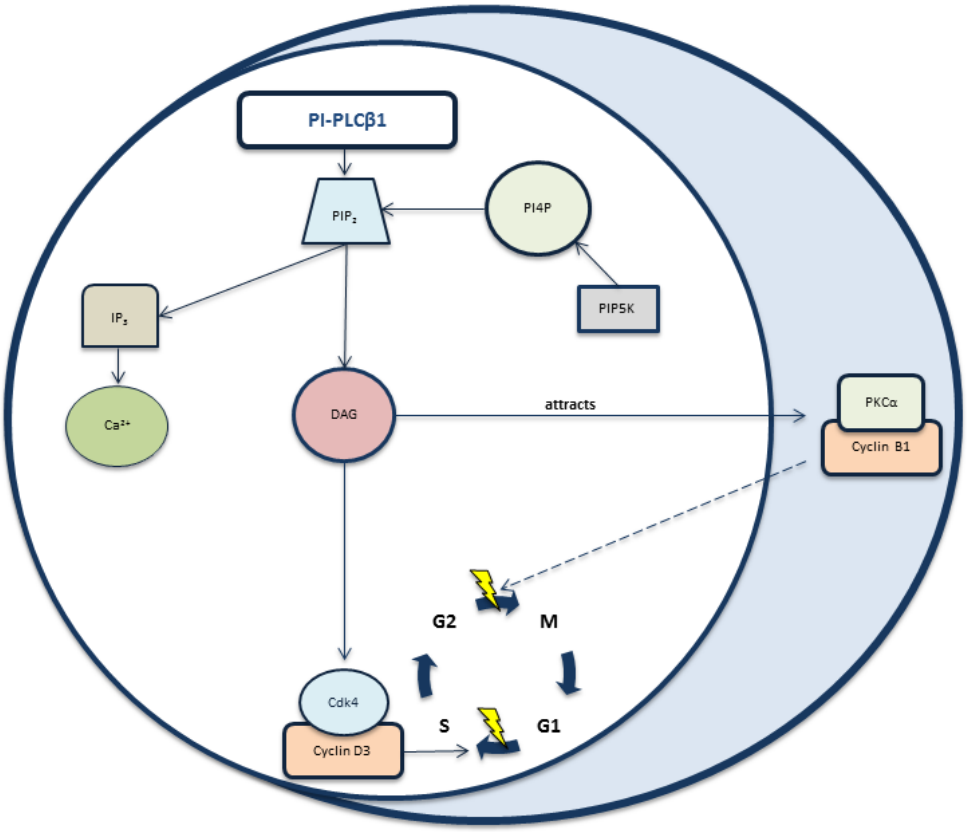
PI-PLCβ1
PIP2
PI4P
PIP5K
IP3
Ca2+
DAG
attracts
PKCα
Cyclin B1
G2
M
Cdk4
Cyclin D3
S
G1

Figure 1

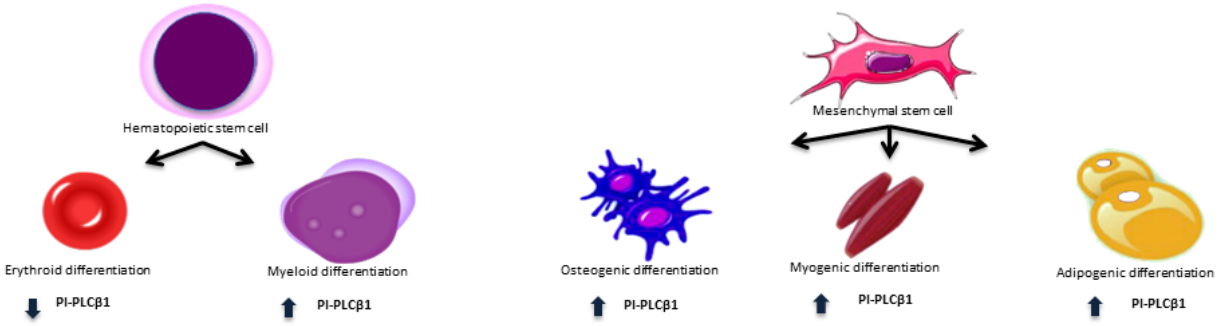
Hematopoietic stem cell
Erythroid differentiation
PI-PLCβ1
Myeloid differentiation
PI-PLCβ1
Osteogenic differentiation
PI-PLCβ1
Mesenchymal stem cell
Myogenic differentiation
PI-PLCβ1
Adipogenic differentiation
PI-PLCβ1

Figure 2