Chytła *et al. Lipids in Health and Disease* (2025) 24:39
https://doi.org/10.1186/s12944-025-02452-6

Lipids in Health and Disease

REVIEW Open Access

Plasma membrane and nuclear phosphatidylinositol 4,5-bisphosphate signalling in cancer

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Abstract

The development of metastasis is a leading cause of cancer-related death that involves specific changes in the plasma membrane (PM) and nucleus of cancer cells. Elevated levels of membrane lipids, including sphingomyelin, cholesterol, and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), in the PM, contribute to changes in membrane rigidity, lipid raft formation, and actin polymerisation dynamics, processes that drive cell invasion. This review discusses the relationship between well-studied cytoplasmic phosphoinositides and their lesser-known nuclear counterparts, highlighting their functional role in metastatic progression. Nuclear phosphoinositides, particularly PI(4,5)P2, are essential for regulating transcription factors and chromatin organisation, thereby shaping gene expression patterns. We also explore the role of PI(4,5)P2 and its metabolism in cancer cell invasiveness and metastasis, proposing a model in which the dysregulation of cytosolic and/or nuclear PI(4,5)P2 pool triggers malignant transformation. Understanding the PI(4,5)P2-related mechanisms underlying metastasis may provide insights into potential therapeutic targets, paving the way for more effective therapies and improved patient outcomes.

Introduction

Cancer is a leading cause of death worldwide [1]. In most cases, the increased invasiveness of cancer cells is responsible for their lethal potential. Metastasis is defined as the spread of cancer cells from their primary site to neighbouring tissues and other organs via blood vessels and the lymphatic system. This process can be divided into five sequential stages. Invasion, a crucial step in the progression of metastasis, followed by intravasation, survival in circulation, extravasation, and colonisation [2]. A deeper understanding of the molecular mechanisms underlying this metastatic cascade will lead to the development of effective therapies and improved patient prognosis.

Metastatic progression is associated with altered composition and dynamics of the plasma membrane (PM) and cell nucleus. Given the complex nature of the PM, consisting of a lipid bilayer and various proteins, these alterations can occur as changes in membrane rigidity, increased lipid raft formation, or differences in PM signalling [3], as well as alterations in actin polymerisation and depolymerisation, which contribute to enhanced cellular invasion [4]. Lipid rafts are highly ordered microdomains enriched in sphingomyelin and cholesterol [5], serving as key platforms for physiological processes, including signalling, migration, trafficking, cellcell communication, and cellmatrix interaction [6]. Although the enrichment of sphingomyelin and cholesterol mainly characterises lipid rafts, significant amounts of

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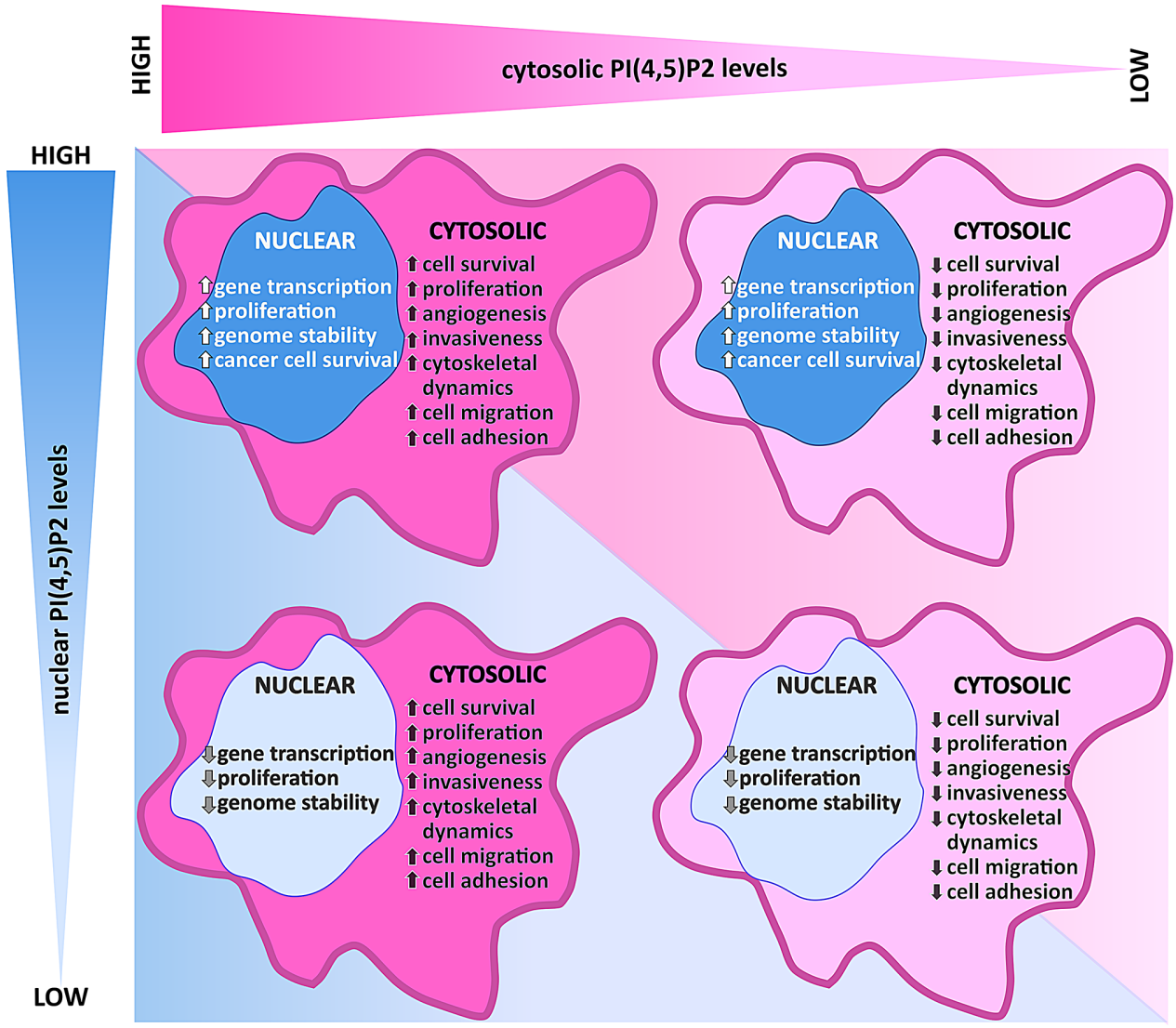
Graphical Abstract



Keywords Phosphatidylinositol 4,5-bisphosphate, Nucleus, Biocondensates, Cancer, Metastasis, HPV

phosphoinositides (PIPs) are also present [7, 8]. Among these, phosphatidylinositol 4,5bisphosphate (PI(4,5)P2), is a crucial regulator of Factin dynamics, significantly influencing cell dynamics and invasive potential [9]. Disruption of lipid rafts within the PM, responsible for organising specific proteins and regulating lipid microdomains, can modulate membraneassociated signalling pathways, such as the phosphoinositide 3kinase/protein kinase B (PI3K/Akt) pathway [3, 10]. This, in turn, affects the migratory and invasive behaviour of cancer cells by altering actin remodelling, promoting invadopodia formation, and enhancing their capacity to degrade the extracellular matrix (ECM). Ultimately, these changes increase the metastatic potential of cancer cells [4].

In addition, nuclear dynamics affect cancer metastasis by altering the shape and size of the nucleus and the structure of the nuclear envelope [11, 12]. Studies have demonstrated that nuclear morphological changes are prevalent in cancer cells and may influence their invasiveness. For instance, cancer cells frequently exhibit enlarged and irregularly shaped nuclei, which have been linked to an elevated capacity for metastasis in a multitude of distinct cancer types [11, 12]. Moreover, dysregulation of nuclear signalling pathways, including those mediated by transcription factors, chromatin modifiers,

and DNA damage response proteins, can significantly affect gene expression patterns involved in cancer metastasis. Transcription factors play a critical role in the regulation of gene expression associated with cell motility, invasion, and metastasis [2]. Dysregulated expression or activity of these transcription factors can promote the expression of prometastatic genes while inhibiting the expression of genes involved in tumour suppression and cell adhesion [2]. Furthermore, alterations in chromatin structure and epigenetic modifications can also contribute to cancer metastasis by affecting gene expression patterns [13]. Epigenetic modifications, such as DNA methylation or histone acetylation, have been demonstrated to regulate the expression of genes involved in metastatic progression. For instance, aberrant DNA methylation patterns at the promoter regions of tumour suppressor genes can result in their silencing, thereby promoting metastasis [14].

Nuclear phosphoinositides have emerged as essential regulators of processes involved in metastasis and invasiveness of cancer cells [15–17]. These lipid molecules not only participate in signalling cascades that control gene expression and cellular responses to extracellular signals but also contribute to the regulation of nuclear envelope dynamics and chromatin organisation [18–20]. Recent studies have underscored the importance of nuclear phosphoinositides in modulating the activities of transcription factors and chromatin modifiers involved in metastatic progression [21, 22].

In this review, we focus on PI(4,5)P2, the most abundant phosphoinositide and a key regulator of cell invasion. We highlight the potential implications of the interplay between cytoplasmic and nuclear PI(4,5)P2 pathways in driving cancer invasiveness.

The role of plasma membrane phosphoinositide-associated structures and molecules in cancer progression and metastasis

Lipids, which serve as the backbone of cell membranes, are essential for maintaining the cellular structure. Membrane composition comprises a mix of three main lipid groups: glycerophospholipids (e.g., phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine), sphingolipids (e.g., sphingomyelin), and sterols (e.g., cholesterol) [23, 24]. In addition, membrane dynamics, together with lipid heterogeneity, leads to the formation of specialised domains that selectively recruit or exclude specific proteins. These dynamic sphingomyelincholesterolrich microdomains, known as lipid rafts, play a crucial role in membrane compartmentalisation [5, 25–27], and are involved in crucial processes associated with cancer progression and metastasis [28]. Lipid raft marker proteins, such as caveolin-1 and flotillin-1 and – 2, have been shown to regulate key cancer processes. In metastatic prostate cancer, phosphorylated caveolin-1 facilitates autophosphorylation of VEGFR2 and its colocalisation at focal adhesion complexes. This, in turn, stimulates angiogenic signalling, which is crucial for new blood vessel formation and tumour growth [29]. The upregulation of flotillin 2 in gastric cancer cells is required for TGFβinduced epithelialtomesenchymal transition (EMT), which increases cancer cell motility and invasiveness [30]. Moreover, overexpression of both flotillin 2 mRNA and protein in breast cancer is associated with poor prognosis, irrespective of disease stage [31]. Since lipid rafts are not the primary focus of this review, we do not discuss this topic in further detail. Researchers interested in a comprehensive overview of lipid rafts in cancer are referred to a recent review [3].

Apart from receptors, phosphoinositides (PIPs) are among the most important molecules in the plasma membrane that are involved in cell signalling [15, 32]. They enable plasma membrane receptors to transmit and amplify signal received from signalling molecules, such as growth factors or hormones. The most common interacting domains found in PIP binding proteins include the pleckstrin homology (PH) domain, Fab-1, YGL023, Vps27, and EEA1 (FYVE), Phox homology (PX), 4.1 protein, ezrin, radixin, moesin (FERM) domain, and plant homeodomain (PHD) finger domains, as reviewed in detail elsewhere [33–35]. PIPs are distributed asymmetrically between the two plasma membrane leaflets. In the outer leaflet, phosphoinositides are associated with GPIanchored proteins in the lipid rafts. PI(4,5)P2 and other phosphorylated isoforms predominantly localize in the inner plasma membrane leaflet because of their critical roles in numerous signalling cascades [42] and regulatory processes [36, 37]. The localisation of PI(4,5)P2 in the inner membrane is significantly affected by the surrounding lipid environment. Lipid domains comprising sphingomyelin with shorter acyl chains provide a stable environment for PI(4,5)P2, promoting its accumulation. Conversely, when the membrane domains are enriched in sphingomyelin with long acyl chains, PI(4,5)P2 is expelled from these domains and instead localises to the less ordered membrane regions [7].

Impact of phosphoinositide imbalance on cancer progression

Two classes of enzymes, kinases and phosphatases, are involved in maintaining the phosphoinositide balance. The class I kinases phosphatidylinositol-4phosphate 5-kinases (PIP5K, PI4P 5-kinase) and class II kinases phosphatidylinositol 5phosphate 4kinase (PIP4K, PI5P 4-kinase) catalyse the phosphorylation of PI4P and PI5P, respectively, to PI(4,5)P2 [32, 37, 38]. PI(4,5)P2 is also a substrate of secondary messengers diacylglycerol (DAG) and inositol trisphosphate (IP3), which are formed

during PI(4,5)P2 hydrolysis by phospholipase C (PLC). The hydrolysis of PI(4,5)P2 to PI4P is catalysed by phosphoinositide 5phosphatases (e.g. Synaptojanin-1, Synaptojanin-2, inositol polyphosphate-5phosphatase B/E/J/K, OCRL inositol polyphosphate-5phosphatase, SHIP2) [32, 37]. These phosphatases, particularly Synaptojanin-2 and SHIP1/2, also catalyse the hydrolysis of PI(3,4,5)P3 into PI(3,4)P2.The formation of PI(3,4,5)P3 from PI(4,5)P2 is catalysed by class I PI3K kinases [17, 32, 39], while the antagonistic reaction is mediated by the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [28, 40]. PTEN is a tumour suppressor protein and lipid phosphatase. Depending on its posttranslational modifications, PTEN is targeted to the nucleus or the plasma membrane [41]. Dysfunction of either kinases or phosphatases causes an imbalance between phosphoinositide levels in the cell and is frequently observed in many diseases, including obesity, type 2 diabetes, endometriosis, neurodegenerative diseases, and cancer [42]. Cancer cells frequently exhibit an imbalance in the levels of phosphoinositides, with a notable increase in the total cellular levels of PI(3,4,5)P3 and a decrease in the total cellular levels of PI(4,5)P2. This imbalance is largely attributed to the loss or inactivation of PTEN or activating mutations in the gene *PIK3CA*, both of which result in elevated levels of PI(3,4,5)P3 [42, 43]. It is still unknown whether these changes affect the cytoplasmic and nuclear PI(4,5)P2 pools in the same way or if they differ from each other. Research suggests that both the cytoplasm and nucleus maintain distinct pools of PIP enzymes, yet some precursors may translocate between these two compartments [44].

PIK3CA encodes the PI3Kα catalytic subunit p110α and is one of the most frequently mutated oncogenes in various types of cancer [44]. The regulatory subunit of PI3K, p85, is encoded by *PIK3R1* (isoforms p85α, p55α, and p50α), *PIK3R2* (isoform p85β), and *PIK3R3* (isoform p55γ). The PI3K signalling pathway is regulated by Src kinase through two mechanisms. Firstly, by direct phosphorylation of the p85 subunit, which recruits PI3K to the plasma membrane, where it can interact with PI(4,5)P2 [45, 46]. Secondly, Src inhibits phosphatase activity and reduces PTEN stability, affecting its ability to hydrolyse PI(3,4,5)P3 [47]. Src, a member of the nonreceptor proteintyrosine kinase family, controls a variety of cellular processes, such as differentiation, proliferation, and adhesion, through a complex regulatory mechanism [48]. During this process, increased Src affinity to the plasma membrane regulates cell motility [49]. Moreover, elevated Src activity has been detected in numerous cancer types, such as skin, ovarian, lung, and head and neck cancers [48, 50]. In squamous cell carcinomas (SCC), including those originating from the head and neck region (HNSCC), lung, vulva and uterine cervical mucosa, an increase in the copy number of chromosome 3q is frequently observed, leading to the amplification of *PIK3C*A [51–53]. The Cancer Genome Atlas for HNSCC revealed that *PIK3CA* is the most frequently altered gene [54]. However, a significant association between *PIK3CA* mutation status and overall survival or diseasefree survival was not observed. Therefore, the clinicopathological and prognostic significance of mutated *PIK3CA* in SCC tissues remains under debate [55]. In smokingassociated HNSCC, universal lossoffunction TP53 mutations and CDKN2A inactivation have been detected, whereas, in HPVassociated HNSCC, mutations in the helical domain of the oncogene *PIK3CA*, loss of TRAF3, and amplification of the cell cycle gene E2F1 are frequently observed [54]. Importantly, in HPVpositive patients, wildtype *PIK3CA* was associated with a 4.64fold increase in the risk of recurrence or death. This implies that stratifying patients according to their HPV status followed by their *PIK3CA* status can help predict prognosis and identify patients who may benefit from deintensified treatment combined with targeted therapies against the affected signalling pathway. In the future, it might be worth considering not only to investigate the mutation per se but also to determine the cellular effects, such as altered cytoplasmic and/or nuclear quantity and localisation of PI(3,4,5)P3, PI(4,5)P2 and other phosphoinositides [56].

Phosphoinositide-mediated actin remodelling in cancer progression

High levels of PI(3,4,5)P3 activate PH domain effector proteins (e.g. Akt, Cdc42, Rac) and downstream signalling involved in processes, such as cell growth, proliferation, survival, and metabolism, which can lead to increased invasiveness, metastasis, and resistance to therapy in cancer cells. Metabolic dysregulation caused by the increased hydrolysis of PI(4,5)P2 by PLC leads to increased production of inositol trisphosphate (IP3) and diacylglycerol (DAG). This process triggers the release of calcium ions (Ca2+) from intracellular stores, which subsequently activates protein kinase C (PKC). As a result, various signalling pathways, including RAS/RAF/MAPK/ERK and JAK/STAT, are initiated, playing key roles in the regulation of angiogenesis, cell proliferation, survival, and autophagy [2, 39]. Furthermore, the organisation of the actin cytoskeleton is influenced by the PI(4,5)P2 and PI(3,4,5)P3 metabolism. The actin cytoskeleton is a dynamic structure of actin filaments responsible for maintaining cell shape, vesicle trafficking, cell division, signalling, and invasiveness (Fig. 1). PI(4,5)P2, which transiently regulates actinbinding proteins (ABPs), is responsible for actin cytoskeletal dynamics by regulating the formation of actin filaments (Factin) from monomeric actin molecules (Gactin) [9]. The neuronal WiskottAldrich syndrome protein (NWASP) is recruited to the cell

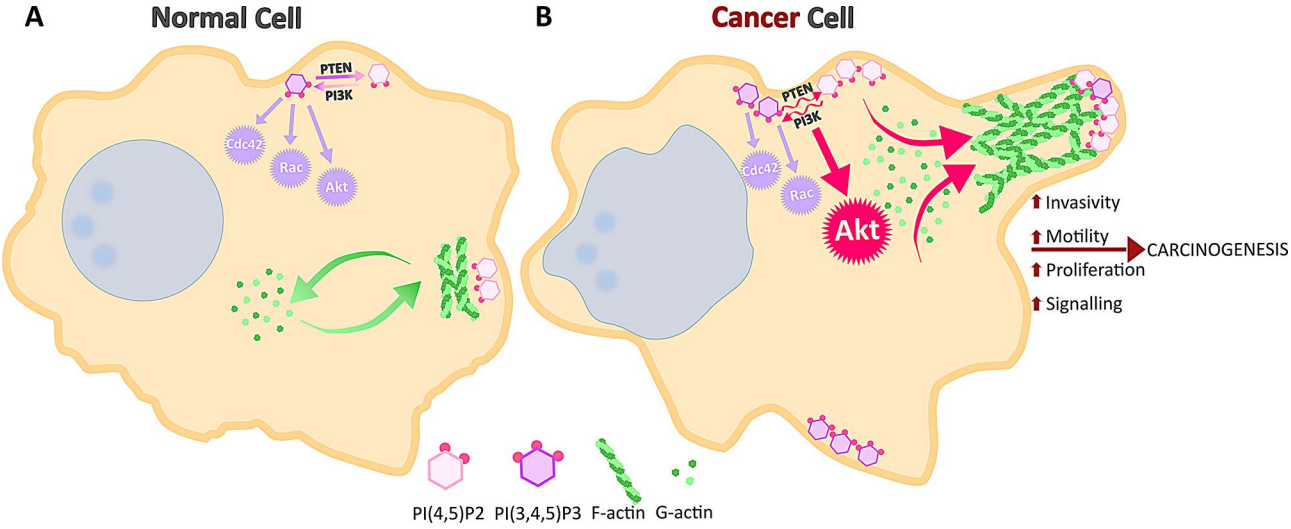


Fig. 1 The dynamic regulation of the cytosolic PI(4,5)P2/PI(3,4,5)P3 levels plays a crucial role in many key cell processes, including cell growth, proliferation or organisation of the actin cytoskeleton. The figure illustrates how the disruption of PI(4,5)P2 homeostasis modulates PI3K/Akt downstream signalling and actin cytoskeleton dynamics. (**A**) In normal cells, the two actin forms, monomeric actin (Gactin) and filamentous actin (F-actin) are under constant dynamic conversion driven by PI(4,5)P2 via actin regulatory proteins. (**B**) In cancer cells, the imbalanced PI(4,5)P2/PI(3,4,5)P2 levels influence the hyperactivation of Akt. This, in turn, leads to alterations in actin remodelling dynamics, which can be observed as increased actin polymerisation, invadopodia formation and, in consequence, enhanced cell motility, invasivity and metastasis

membrane by PI(4,5)P2. It changes its conformation and activates the actinrelated protein 2/3 (ARP2/3) complex, which exhibits nucleation and branching activities. Binding to PI(4,5)P2 inhibits profilin activity, which delivers monomeric Gactin to the barbed ends. Similarly, gelsolin and cofilin severing activities were blocked by PI(4,5)P2. Active gelsolin has an additional capping ability that blocks the free ends of actin. In contrast, once dissociated from the membrane, active cofilin accelerates the dynamics of actin turnover through severing and depolymerisation activity [37]. Cofilin activity is dependent on phosphorylation, a process that is tightly regulated by RhoGTPase, LIM kinase (LIMK), and phosphoinositide binding. The Rho, Rac, and Cdc42 members of the Rho family of small GTPases play pivotal roles in orchestrating actin reorganisation via their diverse downstream effectors [57]. Rho, Rac, and Cdc42 are known regulators of podosomes. Podosomes are actinbased structures that mediate cellmatrix contacts [4]. They consist of more than 300 proteins, including ABPs, such as NWASP, Arp2/3, cofilin, and cortactin. They can be found in different cell types like monocytic cells, osteoclasts, and endothelial cells. They participate in various physiological processes such as immune surveillance, bone degradation, and angiogenic sprouting. These processes facilitate extracellular matrix (ECM) degradation and invasion of the surrounding tissues. In podosomes, the PI3K/Akt signalling pathway and Src kinase promote actin polymerisation and matrix metalloproteinase (MMP) secretion [4, 28]. Similar actinbased and lipid raftenriched structures, with the addition of Diaphanousrelated formins (DRFs), have been identified in highly invasive cancer cells [4]. In this context, the protrusive structures with ECM degradation activity that drive proteolytic invasion are known as invadopodia. They play a crucial role in increased cellular migration, angiogenesis, and metastasis of cancer cells [28, 58]. Similar to podosomes, invadopodia activity and formation are regulated by PI(4,5)P2/PI(3,4)P2 levels, PTEN inactivation, and actin turnover. Moreover, invadopodia formation has also been shown to be lipid raftdependent. In breast cancer cells, colocalisation of the matrix metalloproteinase MT1MMP with caveolin-1 promotes the recruitment of invadopodia to lipid rafts. At the same time, *CAV1* silencing and lipid raft disruption by mβCD were found to inhibit MT1MMP activation and abolish invadopodia formation [59]. In addition, another study demonstrated that caveolin-1 activation of MT1MMP occurs through the PI3K/AKT/mTOR pathway [60].

The role of nuclear phosphoinositides in chromatin regulation, transcription, and cancer progression

The role of actin in the cytoplasm is well known, but its presence in the cell nucleus has been debated for many years. Recently, the development of advanced imaging techniques has made it possible to visualise and study nuclear actin in detail [61, 62]. Actin lacks a nuclear localisation signal (NLS); thus, its nuclear localisation depends on active transport. This shuttle transport is enabled by cofilin and importin 9, which are responsible for importing actin into the nucleus, and profilin and exportin 6, which facilitate actin export [63]. The main

limitations of this process are the availability of Gactin in the cytoplasm and the level of unphosphorylated cofilin. For a long time, the inability to visualise filamentous nuclear actin led to the assumption that actin did not form filaments within the nucleus. However, there is now evidence that nuclear actin filaments can be observed during specific physiological processes or pathological conditions, although they are mainly present as monomers [61, 64]. One of these processes is the regulation of chromatin regulatory complexes. Actin interacts in a PI(4,5)P2dependent manner with the mammalian ATP-dependent chromatinremodelling complex (SWI/SNFlike BAF complex), which is involved in a variety of cellular processes ranging from gene activation and repression to cell development and differentiation. [65, 66]. In homologydirected DNA damage repair (DDR), the N-WASP/Arp2/3 complex mediates nuclear actin polymerisation, which is required for the clustering of DNA breaks [63]. In addition, actin has been reported to be involved in the initiation and regulation of transcription mediated by all three RNA polymerases [67–69]. Additionally, nuclear Gactin plays a role in Pol II pause release through its interaction with PTEFb [70]. Interestingly, upon serum stimulation, Arp2/3 and N-WASP promote actin polymerisation, which plays a crucial role in enhancing Pol II clustering and significantly changing the dynamics of transcription condensates. The larger and more stable clusters transcribe genes associated with survival, proliferation, angiogenesis, migration, EMT, and drug resistance [71].

In comparison to actin, the presence of phosphoinositides in the cell nucleus is less controversial and more widely accepted [72, 73]. Phosphoinositides are located in nucleoplasmic membraneless nuclear structures such as nuclear speckles or nucleoli [74–76]. Recently, researchers have started to explore the potential role of phosphoinositides in regulating nuclear processes. The most abundant phosphoinositide detected in these structures was PI(4,5)P2, which is thought to play a role in the spatial and functional organisation of the nucleus (Fig. 2) [75]. The cell nucleus possesses its own pool of phosphoinositidemetabolizing enzymes, which is distinct from the cytoplasmic pool and thus enables them to control their involvement in nuclear processes specifically [77–79].

The expanding role of PI(4,5)P2 in nuclear architecture and organization

Previously, the dysregulation of the nuclear PI(4,5)P2 pool and its metabolism were overlooked, as the observed changes were more aligned with the total cellular PI(4,5)P2 levels. However, recent exploration of the nuclear PI(4,5)P2 has uncovered its role in the crucial nuclear processes, whose dysregulation is associated with various pathological conditions, including cancer [56, 80, 81]. These include the chromatin remodelling process mentioned above, as well as the acylation and deacylation of histones, RNA processing, DDR, regulation of Pol II transcript activity, premRNA splicing, and polyadenylation [19, 74, 82, 83]. For these processes to function properly, it is essential that appropriate proteins and nucleic acids are concentrated in a specific location. This can be achieved through the formation of biomolecular condensates that concentrate molecules and separate them from

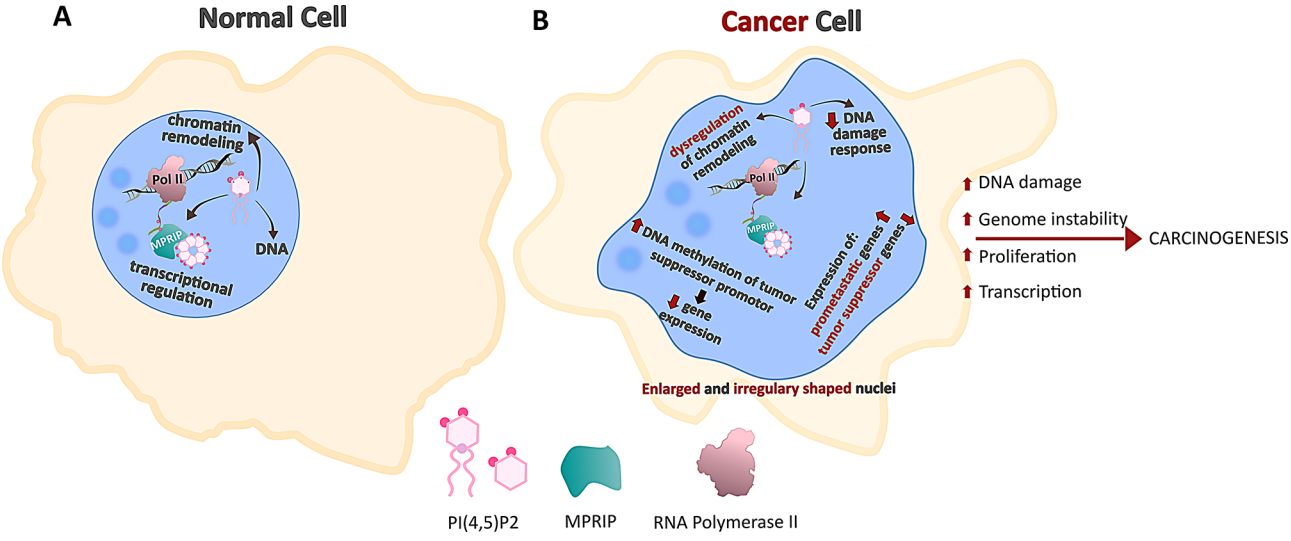


Fig. 2 Schematic illustration of the nuclear PI(4,5)P2 role in normal cells (**A**) and cancer cells (**B**). Figure (**A**) highlights the mechanism of the transcriptional regulation of Pol II in normal cells. The myosin phosphatase Rho-interacting protein (MPRIP) mediates the interaction between Pol II initiation condensate (Tyr1P-CTD) and PI(4,5)P2-containing structures which enables the release of RNAPII from the promoter-proximal pausing. Figure (**B**) highlights the implications of the altered nuclear PI(4,5)P2 levels in carcinogenesis

Table 1 Impact of four potential PI(4,5)P2 deregulation patterns in cancer cells on Cancer Progression and Metastasis

High cytosol levels– High nuclear levels	**Low cytosol levels– High nuclear levels:**
◆ Enhanced cytoplasmic signalling (promoted PI3K/Akt signalling pathway) > increased cell survival, proliferation, angiogenesis, invasiveness. ◆ Dysregulation of actin polymerisation and cytoskeletal remodelling > enhanced cytoskeletal dynamics, cell migration and adhesion. ◆ Promotion of Factin assembly > enhanced migration. ◆ Enhanced invadopodia dynamics (formation and stability) -> ECM degradation. ◆ Enhanced/alternate transcription of genes involved in DNA damage repair, cell proliferation -> promotion of genome stability and cancer cell survival. ◆ Activation of genes involved in cytoskeletal remodelling metastasis	◆ Attenuated PI3K/Akt signalling > impaired cell migration and invasion. ◆ Altered cytoskeletal remodelling > limited dynamics, reduced actin polymerisation, decreased assembly of Factin. ◆ Accumulation of free Gactin molecules > possibility of translocation to the nucleus. ◆ Impaired invadopodia formation. ◆ Activation of signalling pathways involved in the regulation of transcription and DNA damage repair. ◆ Activation of genes involved in cytoskeletal remodelling > metastasis.
High cytosol levels– Low nuclear levels	**Low cytosol levels– Low nuclear levels**
◆ Enhanced cytoplasmic signalling (promoted PI3K/Akt signalling pathway) > increased cell survival, proliferation, angiogenesis, invasiveness. ◆ Dysregulation of actin polymerisation and cytoskeletal remodelling > enhanced cytoskeletal dynamics, cell migration and adhesion. ◆ Promotion of Factin assembly > enhanced migration. ◆ Enhanced invadopodia dynamics (formation and stability) > ECM degradation ◆ Limited/ Attenuated regulation of transcription > altered gene expression profiles. ◆ Decrease nuclear actin levels > impaired chromatin organisation. ◆ Defective DNA repair mechanism > genome instability.	◆ Attenuated PI3K/Akt signalling -> decreased proliferation, cell survival and invasiveness. ◆ Limited cytoskeletal dynamics > reduced motility ◆ Attenuated regulation of transcription -> altered gene expression profiles, decreased invasive potential. ◆ Impaired gene expression. ◆ Decrease nuclear actin levels -> impaired chromatin organisation.

their surroundings [84]. The liquid-liquid phase separation (LLPS) phenomenon plays an essential role in this organisational process [85, 86]. The resulting condensates are usually spherical, dynamically coalesce, and undergo rapid fluorescence recovery after photobleaching, demonstrating a high degree of internal dynamics [87]. The hallmark of the LLPS process is multivalent weak inter and intramolecular interactions, where proteins undergoing LLPS are characterised by distinctive features, such as multiple structured modular domains as well as intrinsically disordered regions (IDRs) or oligomerisation domains [84]. Recently, PIPs have been suggested to be novel LLPS regulatory molecules [22, 74, 88]. The PI(4,5)P2 effector protein and Factin regulator, myosin phosphatase Rhointeracting protein (MPRIP), was identified as a component of the Pol II initiation condensate and as a transcription modulator with condensation capacity [89]. MPRIP recruits myosin 1 C (NM1)/Pol II complex via Pol II Tyr1PCTD to PI(4,5)P2containing structures [21]. Notably, a recent study by Dumilie et al. showed that PI(4,5)P2 is a key component of natural condensates and is enriched in mediator (MED1) condensates [22]. Together with BDR4, MED1 is a transcriptional cofactor found in superenhancers that can compartmentalise the Pol II transcriptional apparatus [90]. The BRD4MED1 SuperEnhancer condensates play crucial roles in boosting the transcription of protooncogenes, which cancer cells use to regulate cell proliferation and survival [91]. Thus, the abnormal LLPS process of PI(4,5)P2containing condensates may lead to the formation of pathological structures with abnormal physicochemical properties and trigger a malignant transcriptional program leading to disease, including cancer progression and metastasis. In addition, the crucial DDR pathway, which is critical for genome stability, may also be affected by abnormal LLPS and contribute to its dysregulation and, ultimately, to carcinogenesis. During the DDR response, the interaction of PI5P, a precursor of PI(4,5)P2, with an inhibitor of growth protein 2 (ING2) leads to the acylation of the tumour suppressor protein p53, thereby transactivating the p53dependent apoptotic pathway [16, 18].

A previously published study showed an increase in PI(4,5)P2 levels in HPV8induced skin tumour samples as well as in HPV16positive samples from lowgrade cervical intraepithelial neoplasia (CIN I) to invasive cervical cancer [92]. HPV E6 protein is known to induce ubiquitination and degradation of the p53 protein [93, 94], while PI(4,5)P2 is known to stabilise p53 [16]. An unknown epigenetic modification may be present in these cancers that protects p53 from degradation and simultaneously ensures cancer cell survival. The elevated nuclear PI(4,5)P2 levels observed in betaHPVpositive skin and HPV16positive cervical tumours highlight the clinical significance of these findings in HPVmediated tumorigenesis. These results established a novel link between HPV infection and nuclear PI(4,5)P2 metabolism. Given that elevated nuclear PI(4,5)P2 is detected as early as in CIN I lesions, HPVinfected cells represent a promising model for studying the role of PI(4,5)P2 in tumour progression and metastasis. The absence of elevated PI(4,5)P2 signals in other skin cancers, such as malignant melanoma and Merkel cell carcinoma, strongly indicates a specific effect on HPVassociated skin tumours [95]. The specificity of these signals warrants further investigation to determine whether similar molecular patterns exist in other cancers or are unique to HPVassociated

cancers. The methodology developed by Hoboth et al. can be used for this purpose on paraffinembedded section patient material obtained from clinicians [96]. Several vital questions must be addressed in future research. First, it is important to investigate whether elevated nuclear PI(4,5)P2 levels are also associated with HPV-16positive oropharyngeal squamous cell carcinoma (OPSCC) compared to nonHPVrelated OPSCC to determine whether the cancer origin and tumour grade affect the elevated nuclear PI(4,5)P2 levels. It also needs to be clarified whether an increase in nuclear PI(4,5)P2 levels is specific to oncogenic skin and mucosainfecting HPV types or if it is a characteristic of HPVinduced tumours or tumours in general. As mentioned above, PI(4,5)P2 is known to localise to nuclear speckles, nuclear lipid islets, and nucleoli [7]. The compartmentalisation of the nucleus and, thus, the existence of different pools of PI(4,5)P2 might allow local finetuning of specific nuclear processes [12]. Using superresolution threedimensional structured illumination microscopy, PI(4,5)P2 was found to localise to nuclear speckles in HPVpositive associated cancers, and the precise link between nuclear speckle function and nuclear PI(4,5)P2 remain elusive and needs to be determined. Furthermore, it should be investigated whether only nuclear PI(4,5)P2 levels are dysregulated in HPVinfected tissues or if other phosphoinositide species are also differentially regulated in HPVrelated tumours. An increase in PI(4,5)P2 may alter PI(3,4,5)P3 levels. However, it remains unclear which nuclear kinases or phosphatases are dysregulated in the presence of the virus. Further research is needed to clarify the mechanisms behind these potential changes in phosphoinositide levels and to understand their implications for the pathogenesis and progression of HPVrelated cancers. Additionally, exploring the interplay between these phosphoinositides may provide insight into early diagnosis, as nuclear PI(4,5)P2 levels changes are observed as early as CIN I or novel therapeutic targets for managing HPVassociated malignancies.

Concluding remarks and prospective directions

Phosphoinositides are fundamental lipids involved in various cellular processes, including the regulation of membrane dynamics and signalling cascades, as well as in nuclear processes such as chromatin organisation. Dysregulation of phosphoinositides, whether in the cytosolic or nuclear pools, can contribute to the progression of several diseases, particularly cancers. Dysregulation of the PI3K signalling pathway has been observed in various types of cancer, underscoring the potential of PI3K as a target for therapeutic intervention. While research involving therapeutic targeting of the PI3K pathway has progressed, the therapeutic efficacy of PI3K inhibitors continues to be challenged by their toxicity, acquired drug resistance, and offtarget activity. Currently, five small molecule PI3K inhibitors have been clinically approved by the U.S. Food and Drug Administration and the European Medicines Agency [97]. Nonetheless, advancement of techniques, such as highthroughput screening, have facilitated the design of novel smallmolecule inhibitors with higher specificity, selectivity, and potency. As a result, more than 60 novel smallmolecule PI3K inhibitors are currently undergoing clinical trials [97].

Recent data suggest that nuclear PI(4,5)P2 plays a crucial role in organising nuclear processes, including RNA processing, DDR, and regulation of Pol II transcript activity [21, 22, 83, 88, 98, 99]. Moreover, elevated nuclear PI(4,5)P2 levels appear to be linked to cancer progression [92]. Studies have also suggested that PI(4,5)P2 is a key component of LLPS biocondensates [22, 88]. However, given that the nucleoplasm is a membraneless environment, a significant question arises regarding the structural form of PI(4,5)P2 condensates. One possibility is that it forms a lipid structure, such as micelles, to hide its hydrophobic acyl chains from the unfavourable environment. Alternatively, the acyl groups of the PIP may integrate into protein condensate structures, forming hydrophobic cores. Another possible scenario is that PI(4,5)P2 may be covalently linked to certain proteins, as shown by Marx et al. [92]. Such conjugation is thought to induce a conformational change in the protein, resulting in the acyl chains being shielded from an aqueous environment. This type of conjugation would represent the novel posttranslational modification of unknown metabolic pathway. Finally, similar to SF-1 protein, certain nuclear proteins might possess hydrophobic binding pockets where they accommodate PI(4,5)P2 acyl chains, leaving the head exposed to the external environment [100]. Therefore, a deeper understanding of the metabolic pathways, structure and functions of nuclear PI(4,5)P2 may provide valuable insights into the mechanisms underlying cancer invasiveness and metastasis.

Signals originating from cytosolic PIPs may also influence nuclear events and vice versa. Some PIPs enzyme precursors are known to shuttle between the nucleus and cytoplasm, allowing for a degree of interconnected regulation [72]. In summary, there are four different patterns in which deregulation of the PIP pathway and its levels can influence cancer progression. The table lists the three with the most likely metastatic ability (high cytoplasmlow nucleus, low cytoplasmhigh nucleus, and high cytoplasmhigh nucleus) and the one with the least invasive ability (low cytoplasmlow nucleus), as it is most likely to contribute to cancer cell death (Table 1). Deciphering the detailed mechanisms of this complex and precise system will not only enhance our understanding of PIPs metabolism in cancers but may also uncover

novel therapeutic targets for malignancies characterised by aberrant nuclear lipid signalling.

Abbreviations

ABD	Adaptor-binding domain
CIN	Cervical intraepithelial neoplasia
DDR	DNA damage repair
ECM	Extracellular matrix
HNSCC	Head and neck squamous cell carcinomas
LLPS	Liquidliquid phase separation
OPSCC	Oropharyngeal squamous cell carcinoma
PI(4,5)P2	Phosphatidylinositol 4,5-bisphosphate
PI3K	Phosphoinositide 3-kinase
PIP4K	Phosphatidylinositol 5-phosphate 4-kinase
PIP5K	Phosphatidylinositol-4-phosphate 5-kinase
PIPs	Phosphoinositides
PM	Plasma membrane
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
RBD	Ras-binding domain
SCC	Squamous cell carcinomas

Acknowledgements
Not applicable.

Author contributions
AC and MS conceptualized the review manuscript. AC prepared the figures and drafted the manuscript. AC, SR, BA and MS wrote and reviewed the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by the National Institute for Cancer Research (NPO EXCELES, ID Project No. LX22NPO5102), Charles University Cooperatio Program: research area "Oncology and Haematology", Fond F Program EXCELENCE1–223011/1LF and the German Research Foundation (AK 42/10 – 1).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 22 November 2024 / Accepted: 27 January 2025
Published online: 06 February 2025

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