Trends in Biochemical Sciences

CellPress

Opinion

PI3K functions as a hub in mechanotransduction

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Mammalian cells integrate different types of stimuli that govern their fate. These stimuli encompass biochemical as well as biomechanical cues (shear, tensile, and compressive stresses) that are usually studied separately. The phosphatidylinositol 3-kinase (PI3K) enzymes, producing signaling phosphoinositides at plasma and intracellular membranes, are key in intracellular signaling and vesicular trafficking pathways. Recent evidence in cancer research demonstrates that these enzymes are essential in mechanotransduction. Despite this, the importance of the integration of biomechanical cues and PI3K-driven biochemical signals is underestimated. In this opinion article, we make the hypothesis that modeling of biomechanical cues is critical to understand PI3K oncogenicity. We also identify known/missing knowledge in terms of isoform specificity and molecular pathways of activation, knowledge that is needed for clinical applications.

Highlights

Class I and class II phosphatidylinositol 3-kinase (PI3K) activation is necessary for the rapid intracellular signal transduction of all mechanical stresses (shear, tension, compression); among those, kilopascal-range compressive stress promotes strong engagement of class I PI3Ks.

Class I PI3Ks are upstream activators of the YAP/TAZ pathway; mechanical stress is permissive for their control of YAP/TAZ and targeting of PI3K is a novel strategy to hinder YAP/TAZ oncogenic dependence.

In response to tension, integrins, cadherins, and ion channels undergo conformational changes, which initiates diverse cytosolic signals including hyperactivation of the class I PI3Ks.

The cytoskeleton forms a rigid network that responds to physical forces. Early signal events lead to PI3K activation to activate small GTPases controlling actin cytoskeleton remodeling.

Nutrient availability and metabolic state control the cell's adaptive response to mechanics via PI3Ks; mechanics induce PI3K-dependent rewiring of cell metabolism (class I) and autophagy (class II).

Mechanical stresses and PI3K signaling in cancer

Mechanical stresses (see Glossary) are ubiquitous in nature. Cells can be stretched or compressed, even deformed (Box 1). Solid tumors are a setting where all types of mechanical stresses can be encountered and experienced by cancer and stromal cells. Although precise mapping of the mechanical stress sensed by tumor cells is unknown, techniques are being developed to improve *in vivo* measurements [1]. Additionally, recent experimental efforts have been directed towards the *in vitro* modeling of these stresses to better study them in a dynamic fashion. Microfluidic devices are appealing systems for exquisite temporal control of both mechanical and chemical conditions [2] (Table 1), as they allow one to reconstitute the biomechanical environment of cancer cells.

In our opinion, it is critical to model these biomechanical contexts and study their cellular integration with biochemical cues, as they might change cancer cell oncogenicity or response to therapy. We feel it is particularly important in the frame of PI3K oncogenic signaling, as recent evidence highlights its role as a key signaling hub in **mechanotransduction**. We also explain why these experimental strategies are critical to improving cancer therapies, especially when using PI3K small-molecule inhibitors that are currently approved or in clinical trials [3]. To support this opinion, we present evidence that PI3K enzymes are early intracellular targets that transduce mechanical stresses into biochemical signals.

Mechanosensing and downstream activation

One of the major challenges in the field remains the understanding of how a mechanical stress is transduced into a biochemical signal leading to a cellular phenotype, and numerous efforts have been made to decipher sensing mechanisms resulting from the modulation of stiffness.

The generic biophysical modifications associated with changes of stiffness are increases in membrane and cortex tension [4]. This increase has been shown to trigger, in particular,

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Box 1. Shear/tensile/compressive stresses

Cells are subject to external mechanical stimulation that can greatly modify their behavior. Different types of mechanical forces can be exerted on cell membranes during tumor progression, such as shear, tensile, and compressive forces. Stress is a measure of force per unit area (expressed in N/m^2, or pascal, like pressure); the applied force is divided by the cross-sectional area that supports the load. Stress must not be confounded with the strain, ε, which measures the relative deformation of a cell induced by an applied stress.

When the force is parallel to the surface (tangential), the stress is called 'shear stress' (Figure I, left). In tissue and in solid tumors, shear stress mainly originates from elevated interstitial fluid pressure, where the gradient of fluid velocity results in viscous friction on the cell surface.

When the force is perpendicular (normal) to the surface and is directed away from the part on which it acts, it is called 'tensile stress', generally described in the range of tens to hundreds of kilopascals, and tends to stretch or elongate cells (Figure I, middle). Tensile stresses emerge from cell adhesion where adhesive proteins are connected to the contractile cell cortex such that cells on a stiffer substrate experience and generate greater contractility of the actin cytoskeleton, leading to higher plasma membrane/cortex tension.

When the force is applied normally to the cell surface and is directed towards the part on which it acts, it is called 'compressive stress', generally described in the range of tens of kilopascals (Figure I, right). Compressive stress does not necessarily require cell adhesion. As cells grow and divide in a limited space, they push against their surrounding environment to accommodate space for new biomass. Cells act on the ECM, on adhesive and cohesive bonds, and against steric constrains due to confinement, resulting in growth-induced compressive mechanical stress. In tumors, the deposition of negatively charged hyaluronic acid results in ECM swelling; this deposition increases the compressive forces on tumor cells, resulting in stroma-induced compressive stress.

These different forces are known to be typically of the order of magnitude of the kilopascal [63,64].

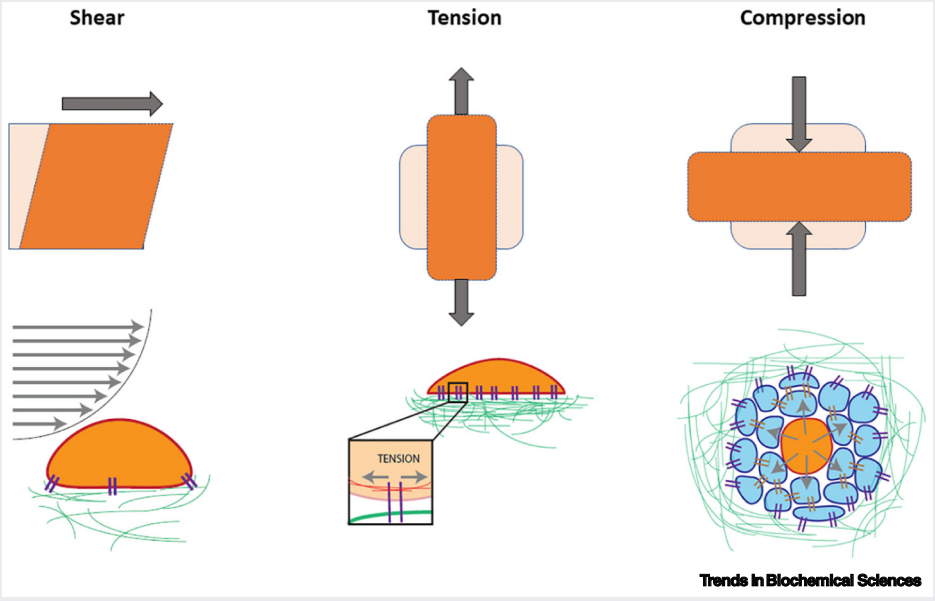

Figure I. Graphical representation of the different mechanical stresses applied on cells. Shear, tensile, and compressive stresses may be applied and participate in mechanical signaling in cells. Orange/blue spheres, cells; green lines, extracellular matrix as collagen fibers; gray arrows, applied forces.

mechanosensors such as the stretch-activated ion channel Piezo1 [5,6] and the well-described **mechanosensitive** transcription factors YAP/TAZ [7], connected to the Hippo pathway [7], pathway named **Hippo/YAP/TAZ**. In addition to this canonical pathway activated with increased stiffness, the PI3K pathway has an important role in signal transduction and is hyperactive in

cancer cells. Because of its positioning at the plasma membrane as a first molecule recruited to membrane receptors such as **receptor tyrosine kinases (RTKs)**, **G protein-coupled receptors (GPCRs)**, or **integrins**, PI3K is a key sensor of extracellular changes whether they are chemical, mechanical, or both and is usually associated with cell cytoskeleton modulation. The oncogenic mutation of the **small GTPase** RAS induces spontaneous **actin cortex** contractility through increased PI3K activity [8]. The changes in tension can also be cell autonomous (i.e., without cell adhesion), through increased tensile forces in the actin contractile cell cortex [9].

Investigations of changes in tension led to the discovery that mechanical stresses impact numerous physiological aspects, from cell proliferation or apoptosis to migration, epithelial-to-mesenchymal transition, even cell differentiation, and all the way to potential drug resistance. Every type of mechanical stress will notably impact membrane tension or the cytoskeleton [5,10]. Recent evidence suggests that other biophysical properties of cells, such as macromolecular crowding or nucleus deformation, can be modulated by compression and may participate in mechanics-induced signaling [11–15]. These elements suggest that **Piezo family** and Hippo/YAP/TAZ signals are not the only pathways involved in mechanical stress sensing and we believe they support the role of the PI3K pathway in primary mechanosensing and its interconnection with well-established mechanical pathways (Figure 1, Key figure).

Mechanical cues activate class I PI3K in cancer

It is well established that reversible phosphorylation of plasma membrane inositol lipids controls diverse functions in cells and that this phosphorylation by PI3K allows a cascade of phosphorylation events downstream, including phosphorylation and activation of AKT and the subsequent activation of mTOR in the mTORC1 complex [16] (Figure 1A). This signaling pathway is involved in the activation of cell growth, proliferation, anchorage, migration, and metabolism and controls **autophagy**. In many cancers, this pathway may be overactive, thus disrupting normal physiological functions.

Although the PI3K family of enzymes can be grouped into three classes (I–III) based on their primary structure, regulation, and *in vitro* lipid substrate specificity [16] (Box 2), PI3K activity usually refers to class I PI3Ks and is the primary focus here. Class I PI3K comprises four enzymes with nonredundant functions [16,17] and nonredundant roles in cancer [3]. The main product of class I PI3Ks is phosphatidylinositol 3,4,5-trisphosphate (PtdIns-3,4,5-P3 or PIP3), generated from phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P2 or PIP2). Class I PI3K activity is stimulated by various types of receptors; for example, PI3Kα/δ are activated by RTKs and PI3Kβ/γ by heterotrimeric GPCRs [18]. Recent studies have also hinted at the role of PI3K in mechanosensing and early biomechanical signal transmission in response to tension [19,20], stretching [21,22], and compression [23,24] of the plasma membrane, as well as shear stress [25–29]. Mechanosensors appear to activate the PI3K/AKT pathway via mostly unknown, precise mechanisms [30].

We know that an increase in PI3K activity represents one of the hallmarks of cancer [8,16]. The importance of PI3K activity in mechanotransduction in cancers is, in our opinion, so far misestimated. Although genetic mutations and epigenetic alterations of class I PI3K can result in its overactivation, we believe, too, that the mechanical context could be permissive for its activation in the absence of oncogenic PI3K mutants. Although a bioinformatics analysis with transcriptomic data from compressed (breast) cancer cells available to date [24] could be used to support this hypothesis, recently published evidence from Kalli *et al.* shows that, among all protumoral signaling pathways, class I PI3Ks appear to be critically involved in the adaptive response to mechanical stresses (Figure 1B) [23]. One of their most striking experiments used phosphorylation screening to assess, in an unbiased manner, the cell signaling response after mechanical stress. With this strategy, they identified that compressive stress strongly activates

Glossary

Actin cortex: a thin, contractile layer of filamentous actin, myosin motors, and regulatory proteins beneath the plasma membrane crucial to cytokinesis, morphogenesis, and cell migration.
Autophagy: allows the orderly degradation and recycling of cellular components. Phosphatidylinositol 3-phosphate (PI-3-P) controls autophagy initiation.
Basal surface: at the basal surface, the basement membrane is a thin layer of ECM that provides cell and tissue support.
Cell–cell adherens and tight junctions: adherens junctions and tight junctions provide important adhesive contacts between neighboring epithelial cells. The classical E-cadherins are major transmembrane proteins of adherens junctions and initiate intercellular contacts through *trans*-pairing between cadherins on opposite cells. E-cadherins are necessary for the determination of cell polarity.
Extracellular matrix (ECM): an extracellular network of molecules that are key to the organization of each tissue.
Focal adhesions: primary site of force transmission into cells; contain high levels of integrin, vinculin, Talin, kindlin, paxillin, zyxin, α-actinin, vasodilator-stimulated phosphoprotein (VASP), FAK, other phosphotyrosine proteins, and integrin αVβ3 and actopaxin.
G protein-coupled receptor (GPCR): transmembrane receptor whose activation leads to conformational changes and modified coupling with heterotrimeric G proteins. There are two principal downstream signal transduction pathways – the cAMP signal pathway and the phosphatidylinositol signal pathway.
Hippo/YAP/TAZ pathway: the YAP and TAZ transcription coactivators are oncoproteins repressed through their phosphorylation by the tumor suppressor LATS1/2 controlled by Hippo. Dysregulation of the Hippo pathway, resulting in an increase in YAP/TAZ activity, is associated with cancer.
Integrins: transmembrane receptors that contribute to cell–cell and cell–ECM adhesion.
Mechanical stress: during cancer development, changes in the mechanical context are observed. The type of mechanical cues that a tumor cell encounters (which one could call the

the PI3K/AKT pathway, leading to transcriptional regulation of the expression of the growth factor GDF15, which promotes pancreatic cancer cell migration.

Modulation of compressive stress activates PI3K signaling to promote cell migration, proliferation, survival, and possibly drug resistance

Kalli *et al.* showed that compression of pancreatic cancer cells promotes a migratory phenotype via an autocrine loop involving PI3K activation [23]. The coupling of mechanical stress and PI3K/AKT pathway activation was also shown to be involved in the regulation of cell death; activation of class I PI3Ks via adhesive molecules, such as N-cadherin, protects against cell death induced by a wide range of compressive stresses [31]. It should be noted that both of these studies were performed using unidirectional compression of cells that potentially promotes both tensile and compressive stresses (Table 1). The cytoskeleton remodeling differs between 2D and 3D mechanical settings [32]; similarly, the signal responses to mechanical stresses could be different. One of the most used experimental approaches to study the effects of mechanical stresses is to embed individual or groups of cells in inert hydrogels. This 3D embedding spatially confines proliferating cells and isotropically compresses them when they proliferate (Table 1). The resulting growth-induced compression has been shown to prevent cell cycle progression and decrease drug sensitivity [33]. Quicker compressive stress relaxation regulates cell cycle progression through a growth-responsive PI3K/AKT–p27Kip1 signaling axis mediated by stretch-activated channels [34]. Interestingly, the response of cells displaying oncogenic PI3K hyperactivation differed at a higher compression range (16 kPa), suggesting that oncogenic mutations could confer different sensitivities in terms of proliferation adaptability to compressive stress.

We argue that, among the signaling pathways involved in the cellular response to mechanical stress, these experiments converge towards a close link between mechanical stress and PI3K/AKT pathway activation that few studies have underlined until now. The following paragraphs emphasize the role of PI3K in mechanotransduction and highlight the current state/lack of knowledge on the function of PI3K classes/members in mechanotransduction processes.

Class I PI3Ks are upstream activators of the YAP/TAZ transcriptional pathway

Mechanotransduction relates to the conversion of a mechanical stimulus from the environment into a biochemical response [10], and cell adaptation to mechanical stress is so far mainly described in terms of gene expression. The transcriptional activators YAP/TAZ in the Hippo pathway are major controllers of gene expression on mechanical stress [7,9]. Three recent studies place PI3K signaling upstream of mechanically induced YAP/TAZ activation [35–37], positioning PI3K activation as a preceding biochemical event converting tensile cell mechanics into YAP/TAZ activation (Figure 1B).

In one study, tension-induced mechanotransduction via the transmembrane proteoglycan adhesion receptor syndecan-4 and its conformational change induce rapid production of PIP3 and a PI3K-dependent locally increased area of **focal adhesions** [36]. PIP3 production is rapidly induced and necessary for local actin cytoskeleton reorganization and activation of the small GTPase RhoA. How the molecular conformational switch of syndecan-4 directly activates PI3K is not fully elucidated, but the PIP3 produced is locally responsible for the activation of RTKs and integrins through an indirect mechanism; examples of such transmembrane activation by class I PI3K have been previously described [38]. This secondary activation of RTKs and integrins by PIP3 triggers a second wave of signal leading to the modulation of YAP activity and of well-known transcriptional Hippo–YAP target expression [36].

In *Drosophila melanogaster*, an organism that has only one class I PI3K (see Table I in Box 2), PI3K activation and PIP3 plasma membrane production by insulin/IGF (RTK ligands) promote the Hippo

'mechanotype') should be better measured with the aim of controlling these parameters in *ex vivo* settings.

Mechanosensitive: mechanosensitive biomolecules (mechanosensors) undergo a conformational change on the application of such force. A mechanosensitive signaling pathway is triggered by mechanosensors in response to a mechanical modulation of the environment.

Mechanosensor: a protein able to detect mechanical stress. Several sensing mechanisms exist, the most common being mechanically induced conformational change, to expose at intracellular sites protein domains that trigger protein–protein interaction or to open ion channels.

Mechanotransduction: relates to the action of transducing a mechanical signal into a biochemical one.

Piezo family: stretch-activated transmembrane Ca^{2+}-permeable channels.

Receptor/protein tyrosine kinase (RTK/PTK): RTKs such as EGFR and the insulin receptor are endowed with intrinsic PTK activity. Other transmembrane receptors (e.g., integrins) transmit their signals into the cell by coupling to non-receptor PTK such as FAK or Src kinase.

Small GTPases: monomeric GTP-binding proteins commonly found in eukaryotic cells. They play an important role in cytoskeletal reorganization, cell polarity, cell cycle progression, and gene expression.

Table 1. Innovating systems that model biomechanical cues in single (2D) or multicellular (3D) settings[a]

	2D cultures	3D cultures	2D microfluidic devices	3D microfluidic devices
Budget	Low cost	Low cost	High cost	High cost
Design and fabrication	Simple setupsUser friendly	Simple setupsUser friendly	Complex setupsDifficult to use	Complex setupsDifficult to use
Imaging	Simple	Complex	Simple	Complex
Duration of time-lapse experiment	Short term	Short term	Long term	Long term
Chemical control	Static	Static	Dynamic	Dynamic
Mechanical control	Static [23]	Static [65]	Dynamical [66]	Dynamical [67]
Types of mechanical stresses that can be performed	-Shear stress-Tensile stress- Compressive stress	-Shear stress-Tensile stress-Compressive stress (external)- Growth -induced pressure	-Shear stress(can be performed dynamically)-Tensile stress-Compressive stress (can be performed dynamically)-Growth-induced pressure	-Shear stress(can be performed dynamically)-Tensile stress-Compressive stress(can be performed dynamically)-Growth-induced pressure
High throughput	Possible	Possible [68]	Possible [69]	Possible [70]
Addition of sensors	Possible	Possible	Possible	Possible
Recollection of sample	Possible	Possible	Never achieved	Never achieved
Coculture of cells	Possible	Possible	Possible	Possible
Coculture with the micro environment	Limited in 2D	Possible	Limited in 2D	Possible [71]

[a]This table presents an overview of some achievements, requirements, and limitations of 2D and 3D devices (usually commercially available) and 2D and 3D microfluidic devices, which typically are required to be created or reproduced. Such devices can be used for bespoke experiments, thus enabling a wide array of scientific investigations. They can, however, be challenging in production or use.

pathway and YAP/TAZ nuclear localization [37]. With this seminal work, the authors showed that the Hippo pathway could be considered one of the key effectors of PI3K/AKT in tissue-growth control.

In mammalian cells, YAP/TAZ nuclear translocation is known to be a key process in normal and tumoral cell proliferation. In skin cells, inhibitors of downstream effectors of the PI3K/AKT pathway that mimic a decrease in PIP3 production prevent YAP nuclear localization (activated YAP) in keratinocytes [37]. Conversely, skin-specific knockout of the tumor suppressor PTEN (a phosphatase that reverses PI3K activity), which induces increased PIP3 production [39], promotes YAP nuclear localization and dermal cell hyperproliferation [37]. The PTEN-loss-induced excessive proliferation of dermal cells is specifically prompted by PIP3 from the PI3Kβ isoform [40], suggesting that PI3K isoforms may not be equivalent in the transduction of mechanical stresses. Another study corroborates this concept, as *in vivo* only PI3Kβ overexpression (and not PI3Kα overexpression) sensitizes untransformed breast cells to YAP/TAZ-induced oncogenicity [35]. PI3Kα-driven YAP/TAZ activation, possibly through EGF–EGFR–PI3K or focal adhesion kinase (FAK)–Src–PI3K activation sequences [41,42], is not sufficient to promote tumor formation, while PI3Kβ-driven YAP/TAZ activation, possibly through pertussis toxin sensitive GPCR-PI3K activation [18,43], allows tumor formation (Figure 1C). As the constitutive activation of PI3K/AKT alone is not sufficient to activate YAP/TAZ organismal functions [36,37], some additional mechanical stimuli (e.g., stretching) may be further needed, promoting potent YAP activation. All of these elements therefore strongly argue for a differential role of PI3K isoforms in YAP/TAZ oncogenic action.

We are convinced that the biomechanical context influences the output signaling of the PI3K pathway. Taking into consideration genetic alterations that skew cancer cell signaling, the identification

Key figure

Phosphatidylinositol 3-kinase (PI3K) signaling, isoform selectivity, and mechanosensing at the cellular cortex

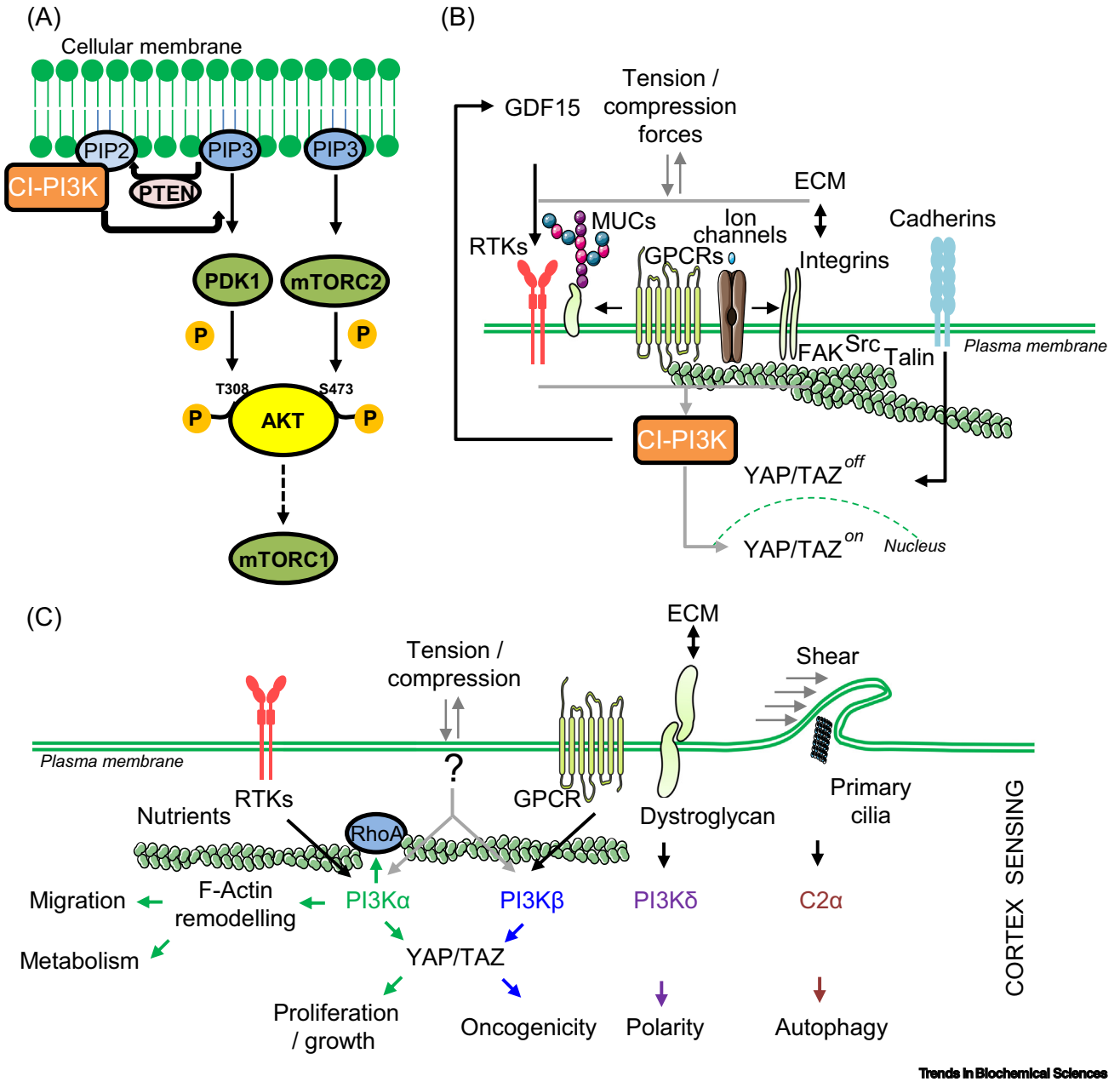

Figure 1. (A) Canonical PI3K–AKT signaling pathway. Class I PI3Ks phosphorylate and transform phosphatidylinositol bisphosphate (PtdIns-4,5-P2, PIP2) into phosphatidylinositol trisphosphate (PtdIns-3,4,5-P3, PIP3). PIP3 induces AKT phosphorylation via PDK1 and mTOR complex 2 (mTORC2), promoting mTOR complex 1 (mTORC1) activation. (B) Mechanical constraints couple to cellular processes at the plasma membrane through two major molecular events: the translation of cortex

(Figure legend continued at the bottom of the next page.)

of which isoform of PI3K integrates both mechanical and chemical cues represents one of the future key challenges.

PI3K activation controls cell cytoskeleton remodeling on mechanical stimulation

Beyond YAP/TAZ transcriptional activation, mechanical cues also trigger cell cytoskeleton remodeling. The latter is critically controlled by focal adhesions and **cell–cell adherens and tight junctions** that mediate bidirectional physical communication between cells and the **extracellular matrix (ECM)**/neighboring cells (Figure 1B).

In focal adhesions, ECM binding to integrin or mucins (e.g., MUC13) activates PI3K activity that enhances the activation of small GTPases such as Cdc42 or RhoA [36,42,44,45]. Hence, the polarization of PIP3 at focal points of the cell membrane activates the actin polymerization/depolymerization cycle by small GTPases and further promotes the formation of focal adhesion at this site [46,47]. PI3Kα is one of the major PI3K isoforms regulating actin cytoskeleton remodeling via Rho GTPase activation [21,48,49].

Stretching of epithelial cells is also sensed by structures that organize polarity and that are linked to the cell cytoskeleton through other molecular links. Polarity is controlled by spatial repartition of the PIP3:PIP2 ratio at the cell membrane. PI3Kδ is the PI3K isoform involved in this process [50] and regulates the organization of specific adhesion sites at the **basal surface** of polarized epithelial cells as well as the localization of their key molecular constituents such as dystroglycan (Figure 1C). Because these structures are disorganized during oncogenesis, it would be interesting to study the importance of PI3Kδ in the sensing of mechanical stretching during this process.

Mode of PI3K activation at focal adhesions

It is generally described that non receptor **protein tyrosine kinases (PTKs)**, such as Src or FAK, are recruited and activated in focal adhesions or at sites of ECM binding to integrin or mucins. By phosphorylating RTKs or adaptor proteins such as p130Cas on YxxM motifs, they can activate PI3K signals (mostly PI3Kα/δ), although the temporality of the activation cascade is not formally demonstrated in these studies [36,42,44,45]. The recent refined description of syndecan-4-induced rapid activation of PI3K activity suggests that this temporality could be not the one that is expected, with PI3K activation being a more upstream signal than currently described.

Mechanical cell transduction can involve class I-, II-, and III-PI3K-dependent regulation of metabolism and autophagy

In the context of mechanically induced contraction of mammalian cells, metabolic reprogramming promotes proinvasive properties [24] (Figure 1C). PI3K coordinates glycolysis with cytoskeletal

membrane tension on the actin cytoskeleton and the coupling of tension with intermediate filaments and microtubules that impact nuclear morphology. Molecular crowding is an emerging mechanism of compression sensing (not shown). Compression, tension, and shear stress converge to modulate cortex tension, referred as cortex sensing. Action on membrane tension activates class I PI3Ks (CI-PI3Ks) via Piezo1 [30], TRPV4 [34], MUC5AC/EGFR [72], MUC13 [45], N-cadherins [54], integrins, and the transmembrane protein syndecan-4. Focal adhesion proteins (FAKs) such as FAK, Src, and Talin [36] control inside–outside signaling [23]. Transactivation between transmembrane receptors (black arrow) is a possible mechanism of PI3K action. Mechanics-activated CI-PI3K promotes the secretion of growth factors that feed forward the signal. Recent evidence shows that the CI-PI3K signal also controls the YAP/TAZ mechanosensor switch to regulate mechanosensitive gene expression. When the Hippo pathway is 'off', the phosphorylated YAP/TAZ is retained in the cytoplasm and may also undergo proteolytic degradation. When the Hippo pathway is 'on', the unphosphorylated YAP/TAZ moves into the nucleus and binds to transcription factors called TEA DNA-binding proteins (TEAD1–4). (C) Known isoform-selective mechanosignaling. Under mechanical stress, PI3Kα is a major activator of RhoA activity, actin cytoskeleton remodeling, and metabolism control, favoring cell migration. Receptor tyrosine kinases (RTKs) activate PI3Kα; G protein-coupled receptors (GPCRs) (that are pertussis toxin sensitive) activate PI3Kβ. While the PI3Kα/YAP/TAZ axis is under the control of nutrient feeding (green arrows), only the PI3Kβ/YAP/TAZ axis is fully oncogenic (blue arrows). Dystroglycan through binding with the extracellular matrix (ECM) organizes cell polarity; recruited PI3Kδs critically control epithelial cell polarity (purple arrows). Shear stress is sensed by the class II PI3K (C2-PI3K) PI3KC2α in primary cilia, where it is specifically located, and is coupled to autophagy (maroon arrows). Only molecular links with mechanical stress that have been demonstrated are shown.

Box 2. PI3Ks and mechanical stress

The PI3K family is involved in primary cell functions such as survival, proliferation, growth, migration, differentiation, protein synthesis, and vesicular trafficking. In humans, the PI3K family is divided into three different classes based on primary structure, regulation, and *in vitro* lipid substrate specificity [16]. The PI3K classes I, II, and III phosphorylate the 3′-position hydroxyl of the D-myo-inositol head group to generate specific phosphoinositide forms [16]. *In vitro*, all classes can generate phosphatidylinositol 3-phosphate [PtdIns-3-P, PtdIns(3)P, PI-3-P], classes I and II can synthesize phosphatidylinositol (3,4)-bisphosphate [PtdIns-3,4-P2, PtdIns(3,4)P2], and only class I can produce phosphatidylinositol (3,4,5)-trisphosphate [PtdIns-3,4,5-P3, PtdIns(3,4,5)P3, PIP3] [73]. Elevated class I PI3K signaling is considered as a hallmark of cancer [74]. The roles of class II and III PI3K in cancer are not fully elucidated.

In vertebrates, the class I PI3K subfamily comprises four members. This class functions as heterodimers with one of four catalytic p110 subunits (p110α, β, δ, or γ) and a regulatory subunit (p85α, p85β, p55γ, p101, or p84) (Table I). The class II PI3K subfamily comprises three catalytic isoforms (C2α, C2β, and C2γ) but, unlike classes I and III, has no regulatory proteins [16]. Class III PI3Ks are the unique PI3K class conserved in all organisms (*Dictyostelium discoideum*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*) and are more similar to class I in structure, as they function as heterodimers of a catalytic (VPS34) and a regulatory (VPS15) subunit. This class is mainly involved in protein and vesicle trafficking [75].

PI3K isoforms were first considered redundant. However, while all synthesize PIPs, each isoform has a particular role; there is also heterogeneity of expression of all classes of PI3K in each cell type or tumor type [16]. Only PI3Kα (encoded by *PIK3CA*) is found mutated in a significant number of cancers (e.g., colon, lung, breast, ovarian); its mutated version is hyperactive and oncogenic. However, the other class I PI3Ks (β, γ, and δ), albeit unmutated, act as oncogenes through their increased activation as well as increased expression.

PI3K isoforms are capable of transducing mechanical stresses. Their specificity of cell activation at the plasma membrane, primary cilia, intracellular vesicles, and nucleus or of their expression in cancer and stromal cells could more precisely be involved in discriminating the mechanotransduction of the three different types of mechanical stress (shear, tensile, compressive stresses) and determining their cellular output. So far, they have been demonstrated to transduce: (i) shear stress – class I [25], class II [26–28] (one study excludes class III PI3K as integrating shear stress, although this was performed in a limited range of model organisms [26]); (ii) tensile stress – class I [21] (roles of classes II and III were not studied); and (iii) compressive stress – class I [23,34,54,65] (the importance of class III PI3K in response to compression is highly suggested by the literature [54], while the role of class II has not been studied so far).

Table I. PI3Ks

Subunit	Protein (human)	Gene name (human)	Gene name (*Drosophila melanogaster*)	Gene name (*Dictyostelium discoideum*)	Gene name (*Saccharomyces cerevisiae*)
Class I					
Catalytic	p110α	*PIK3CA*	*dp110 (DmelPi3K92E)*	*DdPIK1*	
	p110β	*PIK3CB*		*DdPIK2*	
	p110δ	*PIK3CD*		*DdPIK3*	
	p110ϒ	*PIK3CG*			
Regulatory	p85α or p55α or p50α	*PIK3R1*	*dp60(DmelPi3K21B)*		
	p85β	*PIK3R2*			
	p55β	*PIK3R3*			
	p101	*PIK3R5*			
	p84 or p87	*PIK3R6*			
Class II					
Catalytic	PI3KC2α	*PIK3C2A*	*DmelPi3K68D*		
	PI3KC2β	*PIK3C2B*			
	PI3KC2ϒ	*PIK3C2G*			
Class III					
Catalytic	Vps34	*PIK3C3*	*DVps34(DmelPi3K59F)*	*DdPIK5 (Vps34)*	*VPS34p*
Regulatory	Vps15	*PIK3R4*	*Ird1(DmelVps15)*	*DdVps15*	*VPS15p*

dynamics through the control of aldolase localization in an AKT-independent manner [51]. Additionally, it was shown that the resistance of the cytoskeleton in response to mechanical cues enables the persistence of high glycolysis rates in lung cancer cells [52]. Increased PI3K activity in tumor cells could be responsible for cell resistance to antimigratory mechanosensing. During *Drosophila* development, feeding promotes insulin/IGF-1 signaling and PI3K/AKT activity

that is necessary for YAP/TAZ activation by mechanical cues [37]. Hence, mechanically induced PI3K–YAP/TAZ activation in this setting is dependent on nutrient availability.

There is also further evidence that mechanical stress impacts cell metabolism in return. Cells subjected to mechanical stress conditions were shown to mobilize specific membranes and proteins to initiate autophagy. PtdIns-3-P (also referred to as PI-3-P) is produced by class II (PI3KC2α, β, γ) and III (VPS34) PI3Ks and is a crucial lipid in autophagic membrane dynamics (Box 2). Nutrient starvation and the subsequent decrease of PI3Kα/mTORC1 activity and class III PI3K member (VPS34) activation promotes autophagy; autophagy is also triggered by fluid-flow-induced shear stress in endothelial cells. PIK3C2α, not VPS34, localized at the primary cilium participates only in shear-stress-induced autophagy [26] (Figure 1C). In *Dictyostelium discoideum*, an organism with no homolog of vertebrate class II PI3K, compression activates autophagy in an mTORC1-independent manner [53]. It thus remains to be tested in a broader way whether VPS34, PI3Kα/mTORC1, or class II PI3K activity is important in compression-induced autophagy [54] (Box 2).

Novel concepts in mechanics: PI3K signal coupling

In summary, the current evidence argues for isoform-selective roles triggered by specific biomechanical cues. PI3Kα could be linked to tensile and stretching cell adaptation through modulation of the actin cytoskeleton and could control YAP/TAZ activity under nutrient-rich conditions. PI3Kβ could respond to growth-induced compression and loss of organized cell–cell adhesion, being critical for YAP/TAZ activation in those contexts. PI3Kδ could control epithelial cell loss of polarity (Figure 1C). The roles of class I, II, and III PI3Ks in the regulation of mechanically induced autophagy need to be ascertained.

Knowledge of PI3K isoform selectivity in mechanotransduction is important to increase understanding of this important signaling pathway but also to allow clinical application of small-molecule inhibitors. So far, only isoform-selective agents have been approved for cancer treatment [3]. It is interesting to note that the increase of YAP/TAZ transcriptional activity is predictive of the efficacy of the PI3Kβ-isoform-selective inhibitor KIN-193 in cancer [55]. Similarly, E-cadherin is a critical component of normal epithelial cell–cell adhesion and is known to inhibit YAP/TAZ; loss of E-cadherin expression in cancer increases sensitivity to PI3Kβ inhibition [56].

These emerging results convince us that a better dissection of the relationships and molecular mechanisms involved in the integration between genetic alterations activating the PI3K pathway and biomechanical stresses will help to predict responses to therapies targeting these oncogenic pathways. Transcriptomic analysis of compressed cells [24] could reveal evidence of isoform-selective roles of class I PI3Ks under compression using isoform-selective gene signatures [57]. These differential responses in the activation of PI3K isoforms could create valuable therapeutic vulnerabilities that would depend on the specific compressive and genetic contexts in each patient.

The importance of matrix composition (as a factor that modifies tumor mechanics) was studied to screen the efficiency of small-molecule inhibitors in cancer cells; these data improved therapeutic decisions [58]. However, the matrix composition or the presence of cells that produce large amounts of matrix (e.g., cancer-associated fibroblasts) are only one part of cancer mechanics (mostly mimicking tensile stress). It is now crucial to define the real mechanical stress experienced by tumor cells, to model and implement in a dynamic way the three types of mechanical stress on tumor avatars in 3D (Table 1). Given the converging yet differing responses of the three types of mechanical stresses as well as the differential involvement of each PI3K isoform, it is anticipated

that each isoform-selective inhibitor, which is initially designed to target one genetic context (see [3]), might have a different efficiency in the tumor-relevant mechanical context.

Concluding remarks

Mechanotransduction of tensile stress in cancer [59] and the importance of matrix-induced compression in drug delivery through blood vessel clamping [60] are now well accepted concepts in cancer biology. These assumptions led to the development of innovative mechanotherapeutics, currently mostly tested in pancreatic cancer but not validated in humans [61]. Given the recent literature, we are convinced that as-yet-unknown PI3K isoform-selective-induced signals may also play a pivotal role in mechanotransduction and that class I, II, and III PI3Ks merit greater attention as signal integrators of tensile, shear, and solid stress and of biochemical cues (Box 2). More precise positioning of their activation in mechanosignaling is necessary (Figure 1B,C). Isoform-selective PI3K-targeting drugs were recently authorized for the treatment of breast cancers [62]. Further development of basic research in the topic of PI3K and its integration of mechanical cues will lead to the use of these innovative PI3K-targeted agents as a new tool in the arsenal of mechanotherapeutics (see Outstanding questions).

Acknowledgments

We apologize to the authors whose work could not be cited due to limited space. Our work on this topic is funded by Fondation Toulouse Cancer Santé (Mecharesist) and Inserm Plan Cancer (PressDiagTherapy). We thank our colleagues for their critical reading of the manuscript.

Declaration of interests

No interests are declared.

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Outstanding questions

Sensing at the plasma membrane by PI3K: a selective integration of mechanical stresses?

How PI3K oncogenic signaling is intertwined with mechanotransduction at the plasma membrane is poorly described in the cancer setting.

- Do the different types of mechanical stresses (shear, tensile, compressive) induce selective responses controlled by selective isoforms? What is the role of the cytoskeleton?
- Is the integration of mechanical cues, which are now considered major oncogenic signals, driven by the PI3K/AKT pathway? Does the genetic alteration context control the differential response to mechanical stress by PI3K isoforms? Does this create a therapeutic vulnerability?
- Given the increasing evidence for a potent role of YAP/TAZ in malignant tumors, is PI3K signaling also necessary for its activation and could this knowledge be relevant for therapy?
- What is the impact of the metabolic environment and status on mechanical cue integration by class I, II, and III PI3Ks?

Sensing from the cytosol by PI3K: the role of macromolecular crowding?

Macromolecular crowding, which refers to the high concentration of macromolecules in the cytoplasm, can influence the rates of enzymatic reactions in the cell. Crowding is modulated by compressive stresses such as osmotic stress, and is augmented with increased rigidity of the substrate. Crowding at the scale relevant for protein complex reactions is regulated by mTORC1 through ribosome biogenesis. mTORC1 being a downstream effector of PI3K signaling raises the intriguing possibility that mechano-adaptation could occur not only from the cell surface but also from the cytoplasm.

- Does crowding alter PI3K signaling? Does PI3K signaling modulate crowding, and what would be the effect of such modulation?

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- Does PI3K downregulate ribosome biogenesis, increase ribophagy to combat a potential increase in crowding in compressive or tensile settings?