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Manuscript

PI3K functions as a hub in mechanotransduction

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PI3K, cell signaling, mechanotransduction, compression, tension, shear stress

Abstract

Mammalian cells integrate different types of stimuli which govern their fate. These stimuli encompass biochemical (ligands, oxygen, pH) as well as biomechanical cues (shear, tensile, and compressive stresses) that are usually studied separately. The PI3K enzymes, producing signaling phosphoinositides at plasma and intracellular membranes, are key in intracellular signaling and vesicular trafficking pathways. Recent evidence in cancer research demonstrate that these enzymes are essential in mechanotransduction. Despite being a hub in mechanotransduction, the biological and clinical relevance of the integration of both biochemical and biomechanical cues by PI3K-driven signals, especially in cancer cells, is understudied and underestimated. In this Opinion article, we make the hypothesis that modelling biomechanical cues is critical to understand PI3K biochemical signals, highlight the first clues of their involvement in cell mechanobiology, identify missing knowledge in term of isoform specificity and molecular pathways of activation and scrutinize the potential implication of such knowledge in cancer cells.

Definition of mechanical stresses, their ex-vivo 3D cancer modeling, and their connection with PI3K biochemical pathway in cancer

Mechanical stresses are ubiquitous in nature. Cells can be stretched or compressed, even deformed. A stress is defined as a force per unit surface and is measured in pascals (Pa). Mechanical stresses can broadly be separated into two types: tangential and normal [1] (Box 1). Shear stress from fluid flow, coming for instance from elevated interstitial fluid pressure, is a tangential stress, where the gradient of fluid velocity results in viscous friction on the cell surface (Box 1- Figure I left). Tensile and compressive stresses are normal stresses. Tensile stresses typically emerge from cell adhesion, notably through **integrins** which are coupled to **actin contractile cell cortex** such that increase adhesion leads to increase tension of the cell cortex and stretching of the plasma membrane (Box 1- Figure I middle) (see Glossary). Conversely, compressive stress does not necessarily require cell adhesion. It emerges in tumors either from high proliferation in a confined environment (growth-induced pressure) or from the stroma (stroma-induced pressure). As an example, the deposition of negatively charged hyaluronic acid in pancreatic cancer tumors results in extracellular matrix swelling; this increases compressive forces on tumor cells (Box 1- Figure I right). Solid tumors are a setting where all types of mechanical stresses, shear, tensile but also compressive, can be encountered and experienced by cancer and stromal cells. Precise mapping of mechanical stress sensed by tumor cells is unknown; however, tensile stress is generally described to occur in the range of tens to hundreds of kPa while compression is relevant in the tens of kPa. Techniques are developed to improve *in vivo* measurements of mechanical stresses [2]. Recent experimental efforts have also been directed in the *in vitro* modeling of these stresses, to better study them in a dynamic fashion. As such, microfluidic devices appear as appealing systems for exquisite temporal control of both mechanical and chemical conditions [3] (Table 1). They allow to reconstitute the biomechanical environment of cancer cells.

Our Opinion is that it is critical to model these biomechanical contexts and study their cell integration with biochemical cues as they might change cancer cell oncogenicity or response to therapy. It is particularly very important in the frame of PI3K oncogenic signaling as cumulative recent evidences clearly highlight its role as a key signaling hub in mechanotransduction. In our Concluding remarks, we

will explain why these experimental strategies are critical to improve cancer therapy decision, particularly using PI3K small molecule inhibitors that are currently approved or in clinical trials [4]. To support this Opinion, we present evidence that PI3K enzymes are early intracellular targets that transduce mechanical stresses into biochemical signals. We highlight the lack of knowledge to understand the biochemical integration of PI3K oncogenic signaling with oncogenic mechanotransduction at plasma membrane.

Plasma membrane or intracellular mechanosensing and potential PI3K activation

Indeed, 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. Enormous efforts have been placed in deciphering sensing mechanism resulting from modulation of substrate rigidity. However, even if PI3K activation was described in all these studies, the fact that this signaling node is active in most settings was never highlighted nor studied in molecular details.

The generic biophysical modification associated with changes of substrate rigidity also referred as stiffness is an increase in cortex and membrane tension [5]. This increase has been shown to trigger, in particular, **mechanosensors** such as stretch-activated ion channels **Piezo1** [6,7], or the well-described **mechanosensitive** transcription factors **YAP/TAZ** [8], connected to **Hippo** pathway [8] (see Glossary). These changes in tension can also be cell autonomous, through increased tensile forces in actin contractile cell cortex [9]. Investigating changes in tension leads to the discovery that mechanical stresses impact numerous physiological aspects, from cell proliferation or apoptosis, to migration, epithelial-to-mesenchymal transition and even cell differentiation, all the way to potential drug resistance. Every types of mechanical stresses, shear, tensile but also compressive, will notably impact membrane tension or cytoskeleton [1,6]. Recent evidence suggests that other biophysical properties of cell such as macromolecular crowding or nucleus deformation can be modulated in particular by compression. These other biophysical properties may participate in mechanics-induced signaling [10–14] (see Outstanding Questions). We will however focus in this Opinion on mechanosensing from cell surface and shed a light on PI3K signaling in this emerging field of research.

Mechanical cues activate class I PI3K signaling in cancer

It is well established that reversible phosphorylation of inositol lipids controls diverse functions in cells. Increase in phosphatidylinositol-3-kinase (PI3K) activity represents one of the hallmarks of cancer. Usually, PI3K activity refers to class I PI3Ks. However, a total of three PI3K classes are described in vertebrates (Table 2). The main product of class I PI3K is the phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P3), generated from phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P2). This lipid phosphorylation allows a cascade of phosphorylation events downstream, inducing mTORC1 activation *via* **AKT** (Figure 1A). AKT signaling is involved in activation of cell growth, proliferation, anchorage and migration and regulates cell metabolism and **autophagy** (see glossary). Class I PI3K activity is stimulated by various types of receptors (e.g. growth factor receptors) as well as oncogenes (e.g. active mutant KRAS). PI3K α/δ are typically activated by receptors tyrosine kinase **RTKs** and PI3K β/γ by heterotrimeric G protein-coupled receptors **GPCRs** [15] (see glossary).

Some recent studies uncover the relative impact of PI3K in mechanosensing and early biomechanical signal transmission, in response to tension [16,17] and/or stretching [18,19], compression [20,21] as well as to shear stress [22–26]. However, the importance of PI3K pathway in mechanosensing and mechanotransduction in cancers is so far misestimated (Key Figure 1B). Besides, important mechanosensors such as Piezo1 activate PI3K/AKT, through so far unknown mechanism [27]. As described in Table 2, class I PI3Ks contain 4 different enzymes that have non-redundant functions [28] and non-redundant roles in cancer [4]. Only PI3K α (encoded by *PIK3CA*) was found mutated in a significant number of cancers (e.g. colon, lung, breast, ovarian); its mutated version is hyper-active 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.

Beyond the genetic and epigenetic alteration responsible for PI3K/AKT activation, the mechanical context could be permissive for its activation. Mechanical context may suffice to induce the activation of PI3K/AKT canonical oncogenic pathway in the absence of active oncogenic mutant of PI3K α . A

bioinformatics analysis with transcriptomic data of compressed cancer cells (breast) available to date [29] could be used to support this hypothesis, that would need to be formally validated experimentally.

Furthermore, recent published evidence shows that amongst all pro-tumoral signal pathways, class I PI3Ks appears to be critically involved in adaptive response to mechanical stress (Key Figure 1B). This was shown by Kalli *et al* using pancreatic cancer cell lines [21]. One of the most striking experiments consisted in using a phosphorylation screening to assess, in an unbiased manner, cell signaling response after mechanical stress. With this strategy, they identify that compressive stress strongly activates PI3K/AKT pathway leading to a transcriptional regulation of the growth factor GDF15 expression, that promotes pancreatic cancer cell migration [21].

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

Kalli *et al* showed that compression of pancreatic cancer cells promotes migratory phenotype *via* an autocrine loop involving PI3K activation [21]. The coupling of mechanical stress and PI3K/AKT pathway activation is also involved in the regulation of cell death. Activation of class I PI3Ks *via* adhesive molecule such as N-Cadherin protects from cell death induced by high range of compressive stresses [30]. Both these studies were done using unidirectional compression of cells that potentially promotes both tensile and compressive stresses (Table 1). The cytoskeleton remodeling is different under 2D or 3D mechanical settings [31]; similarly, signal response to mechanical stresses could be different. One of the most used experimental approach to study the effect of mechanical stresses is to embed groups of cells or individual cells in inert hydrogel. This 3D embedding spatially confines proliferating cells and isotropically compress them when they proliferate (Table 1). The emerging growth-induced compression has been shown to prevent cell cycle progression and decrease drug sensitivity [32]. Recent data demonstrate that quicker compressive stress relaxation regulates cell cycle progression through a growth-responsive PI3K/AKT-p27Kip1 signaling axis mediated by stretch-activated channels [20]. Interestingly, response of cells displaying oncogenic PI3K hyperactivation was different at higher compression range (16kPa) suggesting that oncogenic mutations could confer different sensitivities in terms of proliferation-adaptability to compressive stress.

Among all signaling pathways involved in cellular response to mechanical stress, these elements converge towards a close link between mechanical stress and PI3K/AKT pathway activation that few studies underlined until now. The following paragraphs will emphasize the role of PI3K in mechanotransduction and highlight the current state/lack of knowledge on the function of PI3K classes/members in mecanotransduction process.

Class I PI3Ks induce a rapid mechanotransduction and are upstream activators of YAP/TAZ transcriptional pathway

Mechanotransduction relates to the conversion of a mechanical stimulus from the environment into a biochemical response (see Glossary)[1]. Cell adaptation to mechanical stress is so far mainly described in term of gene expression. YAP/TAZ is one of the major controllers of gene expression upon mechanical stress. Three recent studies place PI3K signal upstream mechanically-induced YAP/TAZ activation [34–36], positioning PI3K activation as the first biochemical event converting tensile cell mechanics into biochemistry (Key Figure 1B).

Tension-induced mechanotransduction *via* transmembrane proteoglycan adhesion receptor Syndecan-4 and its conformation change induces a rapid production of PtdIns-3,4,5-P3 and a PI3K-dependent local growth of **focal adhesions** (see Glossary) [35]. PtdIns-3,4,5-P3 production is quicker than Talin polymerization and RhoA activation. How the molecular conformational switch of Syndecan-4 activates directly PI3K is not fully elucidated, but the PtdIns-3,4,5-P3 produced binds to Klindin-2 and is locally responsible for activation of RTKs and integrins. A second wave of signal involving an indirect activation of integrins by EGFR leads to modulation of YAP activity, its nuclear translocation and controls mRNA expression of the fibroblast growth factor *CTGF* and of the Ankyrin repeat domain 1 *ANKRD1*, two well-known Hippo-YAP targets [35].

In *Drosophila melanogaster*, organism that only presents one class I PI3K (Table 2), PI3K activation and PtdIns-3,4,5-P3 plasma membrane production by insulin/IGF (RTK ligands) promotes Hippo pathway

and YAP/TAZ nuclear localization [36]. With this seminal work, the authors show that the Hippo pathway could be considered as one of the key effectors of PI3K/AKT in tissue-growth control.

In mammalian cells, if YAP/TAZ nuclear translocation is known to be a key process in normal and tumoral skin cell proliferation, inhibitors of downstream effector of PI3K/AKT were described to prevent basal YAP/TAZ activation in these cells [36]. Conversely, skin-specific knock-out of *PTEN* (phosphatase that reverses PI3K activity) promotes YAP nuclear localization. Of note, in mammals, *PTEN*-loss induced excessive proliferation of derma and is dependent on PI3K β isoform only [37], suggesting that this PI3K isoform could be specifically necessary in YAP/TAZ oncogenic action [36]. Further, others found that, although both PI3K α and PI3K β activate YAP/TAZ and function similarly in breast cancer cells for this matter, co-overexpression of TAZ with PI3K β (encoded by *PIK3CB*) instead of PI3K α (encoded by *PIK3CA*) causes tumor formation in mice *in vivo* [34]. YAP/TAZ pathway-mediated mammary tumorigenesis is critically induced by PI3K β signal [34]. LPA and S1P known to activate PI3K β [15] inhibit *via* their GPCR activation the Hippo pathway kinases Lats1/2, thereby activating YAP/TAZ [38]. EGF-EGFR-PI3K or FAK–Src–PI3K activation of YAP/TAZ was also described in [39,40]. Importantly, the constitutive activation of PI3K/AKT alone is not sufficient to activate YAP/TAZ organismal functions [35,36], some additional mechanical stimulus (*e.g.* stretching) being further needed. It is tempting to speculate that the difference in PI3K α - or PI3K β -driven signal action on YAP/TAZ oncogenicity observed by the authors in *in vitro* clonogenic assay vs. *in vivo* assays [34] can be explained by the differential mechanical context that was imposed in the two assays, hence the importance of reconstituting mechanical context (Table1).

We are convinced that biomechanical context influences the output signaling of PI3K pathway. The implication of these discoveries needs to be investigated in further molecular details in cancer settings, taking into consideration genetic alterations that skew cancer cell signaling. Identifying which isoform of PI3K is integrating both mechanical and chemical cues presents one of the future key challenges to understand the role of class I PI3Ks in mechanosensing to promote tumor progression.

PI3K activation is a major regulator of actin cytoskeleton remodeling upon mechanical stimulation

The focal adhesions, **adherens** or **tight junctions** mediate a bi-directional physical communication between cells and extracellular matrix **ECM** / neighboring cells which critically control cell cytoskeleton (Key Figure 1B) (see Glossary). Focal adhesion, ECM binding to integrin or mucins (*e.g.* MUC13) controlling mechanical stress transduction at the cortex regulate PI3K activity and enhance Hippo pathway or **small GTPase** activation such as Cdc42 or RhoA activation [35,40–42]. The focal adhesions are the primary site of force transmission into cells. Interestingly, the polarization at focal points of cell membrane of PtdIns-3,4,5-P3 (PI3K product) activates actin polymerization / depolymerization cycle and promotes formation of focal adhesion at this site [43,44]. Moreover, PI3K α is one of the major isoforms regulating actin cytoskeleton remodeling *via* Rho GTPase activation [18,45,46].

Stretching of epithelial cells is also sensed by structures that organizes polarity. Interestingly, polarity is controlled by spatial repartition of PtdIns-3,4,5-P3/ PtdIns-4,5-P2 ratio at cell membrane. PI3K δ is the isoform involved in this process [47]. The δ isoform of phosphoinositide 3-kinase co-localizes with focal adhesion proteins at the **basal surface** (see Glossary) of polarized epithelial cells and regulates the organization of focal adhesions and membrane localization of their molecular constituent such as dystroglycan (Key Figure 1C). As, during oncogenesis, these structures are disorganized, it would be interesting to study the importance of PI3K δ in the sensing of mechanical stretching during this process.

It is generally described that non receptor protein tyrosine kinase (**PTK**) 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 PI3K α/δ) although the temporality of the activation cascade is not formally demonstrated in these studies [35,40–42]. 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.

In the context of mechanical-induced contraction of mammalian cells, metabolic reprogramming promote pro-invasive properties [29] (Key Figure 1C). PI3K was found to coordinate glycolysis with cytoskeletal dynamics through the control of aldolase localisation in an AKT-independent manner [48]. Further to these data, it was shown that the resistance of the cytoskeleton in response to mechanical cues enables the persistence of high glycolytic rates in lung cancer cells [49]. Increased PI3K activity in tumor cells could be responsible for cell resistance to anti-migratory mechanosensing.

Mechanical cell transduction involves class I-, II-, III- PI3K dependent regulation of autophagy & metabolism

During *Drosophila* development, feeding promotes insulin/IGF-1 signaling and PI3K/AKT activity, that is necessary for YAP/TAZ activation by mechanical cues [36]. Hence, mechanically induced PI3K-YAP/TAZ activation in this setting is dependent on the 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. Nutrient starvation, the subsequent decrease of PI3K α /mTORC1 activity and class III PI3K member (VPS34) activation promotes autophagy; autophagy is also triggered by fluid-flow induced shear stress in endothelium cells. In mammalian cells, this specific autophagic response depends on primary cilium signaling and leads to cell size regulation. PtdIns-3-P (also referred to as PI-3-P) is produced by Class II (PI3KC2 α , β , γ) and III (VPS34) PI3Ks and it is a crucial lipid in autophagic membrane dynamics (Table 2). PI3KC2 α and not VPS34 was shown to participate only in shear-stress induced autophagy, while VPS34 was clearly required for starvation-induced autophagy [23] (Key Figure 1C). In *Dictyostelium discoideum*, an organism which do not present any homolog of vertebrate Class II PI3K, compression activates autophagy in a mTORC1 independent manner [50]. It thus remains to be tested in a broader way whether VPS34 or PI3K α /mTORC1 or class II PI3Ks activities are important in compression-induced autophagy [51].

Novel concepts in mechanics – PI3K signal coupling

The current evidence argues for isoform selective roles in biomechanical cue context. From the analysis of the current literature, PI3K α could be linked to tensile and stretching adaptation through actin cytoskeleton and would control YAP/TAZ under nutrient-rich conditions. PI3K β could respond to growth-induced compression, being critical for YAP/TAZ activation in this context and PI3K δ is key for the correct control of epithelial cell polarity (Key Figure 1C). The roles of each class I, II, III PI3K in the regulation of mechanically induced autophagy needs to be ascertained. It is interesting to note that the increase of YAP/TAZ gene signature is predictive of KIN-193 PI3K β inhibitor efficacy in cancer [52]. E-cadherin is a critical component of normal epithelial cell / cell adhesion, loss of E-cadherin expression in cancer increases sensitivity to PI3K β inhibition [53]. Transcriptomics analysis of compressed cells [29] could also reveal evidence of isoform-selective roles of class I PI3Ks under compression using isoform selective gene signatures [54]. These differential responses on the activation of PI3K isoforms could create valuable therapeutic vulnerabilities that would depend on the specific compressive and genetic contexts of each patient.

These emerging results convince us that better dissecting the relationship and molecular mechanisms involved in the integration between genetic alterations activating PI3K pathway and biomechanical stresses will help to predict responses to therapies targeting these oncogenic pathways. In this sense, the newly developed tumor avatars (patient-derived multicellular organoids or xenografts) could provide personalized sensitivity profiles and therapeutic decision for each patient. However, as of now, these systems do not completely reproduce the complex biomechanical environment of these tumors [1] or early lesions [2]. This gap in modeling could in part account for the discrepancy between *ex vivo* prediction and clinical read-out. In the same line of thought, the importance of matrix composition was studied to screen the efficiency of small molecule inhibitors in cancer cells; these data improved therapeutic decision [55]. However, matrix composition or presence of cells that produce large amount 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** (see Glossary) experienced by tumor cells, model and increment in a dynamic way the three types of mechanical stress on tumor avatars in 2D or 3D (Table 1). Given the converging yet different responses of the three types of mechanical stresses as well as the differential involvement of each PI3K isoforms, it is anticipated that

each isoform selective inhibitor, that are initially designed to target one genetic context, (see [4]) might have different efficiency in tumor-relevant mechanical context.

Concluding remarks

Mechanotransduction of tensile stress in cancer [56] and importance of matrix-induced compression in drug delivery through blood vessel clamping [57] are now well accepted concepts in cancer biology. These assumptions led to the development of innovative mechanotherapeutics currently mostly tested in pancreatic cancer, yet not validated in humans so far [58]. Given the recent literature, we are convinced that so far unknown PI3K isoform selective-induced signals may also play a pivotal role in mechanotransduction and that class I, II and III PI3Ks merit a stronger attention as signal integrators of tensile, shear and solid stress and of biochemical cues (Table 2), as well as a more precise positioning of their activation in the mechanosignaling. Isoform-selective PI3K targeting drugs were recently authorized for the treatment of breast cancers [59]. 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.

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Glossary

Integrins: are transmembrane receptors that contribute to cell-cell and cell-extracellular matrix (ECM) adhesion. Upon ligand binding, integrins activate signal transduction pathways that mediate cellular signals such as regulation of the cell cycle, organization of the intracellular cytoskeleton, and movement of new receptors to cell membrane.

Actin contractile cell cortex: is a thin layer of filamentous actin, myosin motors, and regulatory proteins beneath the plasma membrane crucial to cytokinesis, morphogenesis, and cell migration. The actin-rich cell cortex is a viscoelastic structure that contributes to membrane tension.

Mechanosensor: is a protein able to detect a mechanical stress. Several sensing mechanisms exist, the most common one being mechanically-induced conformational change, for instance present in integrins or Piezo1.

Piezo family: are stretch-activated transmembrane **Ca²⁺-permeable** channels.

Mechanosensitive: A mechanosensitive signaling pathway is triggered by mechanosensors in response to a mechanical modulation of the environment. Usually, mechanosensitive biomolecules undergo a conformational change upon application of such force, thus exposing functional domains to the extracellular or intracellular environment (e.g. Syndecan-4) or opening channels (e.g. Piezo1). The Hippo pathway is a notable mechanosensitive pathway.

Hippo/YAP/TAZ pathway: The pathway consists of a complex cascade of serine/threonine-protein kinases. Serine threonine kinase 3 (STK3) and STK4 (also known as MST1 and MST2) are the mammalian equivalent of the *Drosophila* Hpo protein. These kinases form a complex with the adaptor protein, salvador homolog 1 (SAV1) to phosphorylate and activate the effector protein, large tumor suppressor 1/2 (LATS1/2). MAP4K and TAOK kinases also act in parallel to phosphorylate LATS1/2. Once activated, LATS1/2 binds MOB kinase activator 1A/B (MOB1A/B) and inhibits the transcription cofactors yes-associated protein (YAP1) and transcriptional co-activator with PDZ-binding motif (TAZ or WWTR1). 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). The YAP/TAZ-TEAD complex regulates proliferative and pro-survival genes. YAP and TAZ

transcription coactivators are oncoproteins repressed by the tumor suppressor LATS1/2. Dysregulation of the Hippo pathway, resulting in an increase in YAP/TAZ activity, is associated with cancer, promoting hyperproliferation, cellular invasion, metastasis, and chemoresistance.

AKT: Protein kinase B (PKB), also known as AKT, is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration. AKT was identified in early genetic and biochemical studies as a main regulator of FOXO function in diverse organisms. Though other FOXO regulatory pathways and mechanisms have been delineated since, AKT remains a key regulator of the pathway. mTOR links with other proteins and serves as a core component of two distinct protein complexes, mTOR complex 1 (mTORC1) and mTOR complex (mTORC2), which regulate different cellular processes. In particular, as a core component of both complexes, mTOR functions as a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy, and transcription. mTORC2 activates AKT in a PI3K-dependent manner and mTORC1 is activated by AKT.

Autophagy: is a natural, regulated mechanism of cells that removes unnecessary or dysfunctional components. It allows the orderly degradation and recycling of cellular components. Phosphatidylinositol-3-phosphate (PI-3-P) is a key player in membrane dynamics and trafficking regulation, also controls autophagy initiation. VPS34 is the enzyme responsible for most PI3P synthesis. VPS34 and proteins such as Beclin1 and ATG14L that regulate PI3P levels are positive modulators of autophagy initiation.

RTK, PTK: are tyrosine kinases. A variety of extracellular signals are relayed intracellularly through activation of protein tyrosine kinases (PTKs). Receptor tyrosine kinases (RTKs) such as EGFR, Insulin receptor are endowed with intrinsic PTK activity. Other transmembrane receptors transmit their signals into the cell by coupling to non-receptor PTK such as focal adhesion kinase FAK or Src kinase.

GPCR: also known as seven-(pass)-transmembrane domain receptors, 7TM receptors form a large group of evolutionarily-related proteins that are cell surface receptors that detect molecules outside the cell and activate cellular responses. Ligands can bind either to extracellular N-terminus and loops or to the binding site within transmembrane helices. They are all activated by agonists although a spontaneous auto-activation of an empty receptor or by cleavage. Activation leads to conformational changes and modified coupling with heterotrimeric G proteins. There are two principal signal transduction pathways involving the G protein-coupled receptors the cAMP signal pathway and the phosphatidylinositol signal pathway.

Mechanotransduction: It relates to the action of transducing a mechanical signal into a biochemical one. For instance, increase rigidity triggering cell proliferation. Mechanotransduction can often trigger cellular signaling processes much faster than a purely chemical means of activation.

Focal adhesions: contain high levels of integrin, vinculin, talin, kindlin, paxillin, zyxin, α -actinin, vasodilator-stimulated phosphoprotein (VASP), focal adhesion kinase (FAK), phosphotyrosine proteins, and integrin α v β 3 and actopaxin. The focal adhesion mechanosensing activity consists in perceiving and transferring mechanical cues arising from the extracellular environment to the cellular cytoskeleton (e.g. binding to differential concentration of matrix).

Extracellular matrix:Cell-cell adherens junctions: are mainly driven by E-Cadherin homotypic binding. These E-cadherins associated with catenin family members including p120-catenin, β -catenin, and α -catenin form the core of the adherens junction and control the formation, maintenance, and function of adherens junctions. Adherens junction and tight junction provide important adhesive contacts between neighboring epithelial cells. The classical E-cadherins are major transmembrane proteins of adherens junction and initiate intercellular contacts through trans-pairing between cadherins on opposite cells E-cadherins are necessary to the determination of cell polarity.

Basement membrane: is a thin layer of extracellular matrix, that provides cell and tissue support. It acts as a platform for complex signaling. The primary function of the basement membrane is to anchor down the epithelium to its loose connective tissue underneath.

Small GTPases: are monomeric GTP-binding protein commonly found in eukaryotic cells. It plays an important role in cytoskeletal reorganization, cell polarity, cell cycle progression, gene expression. Small GTPases also exhibit a remarkable diversity in both structure and function.

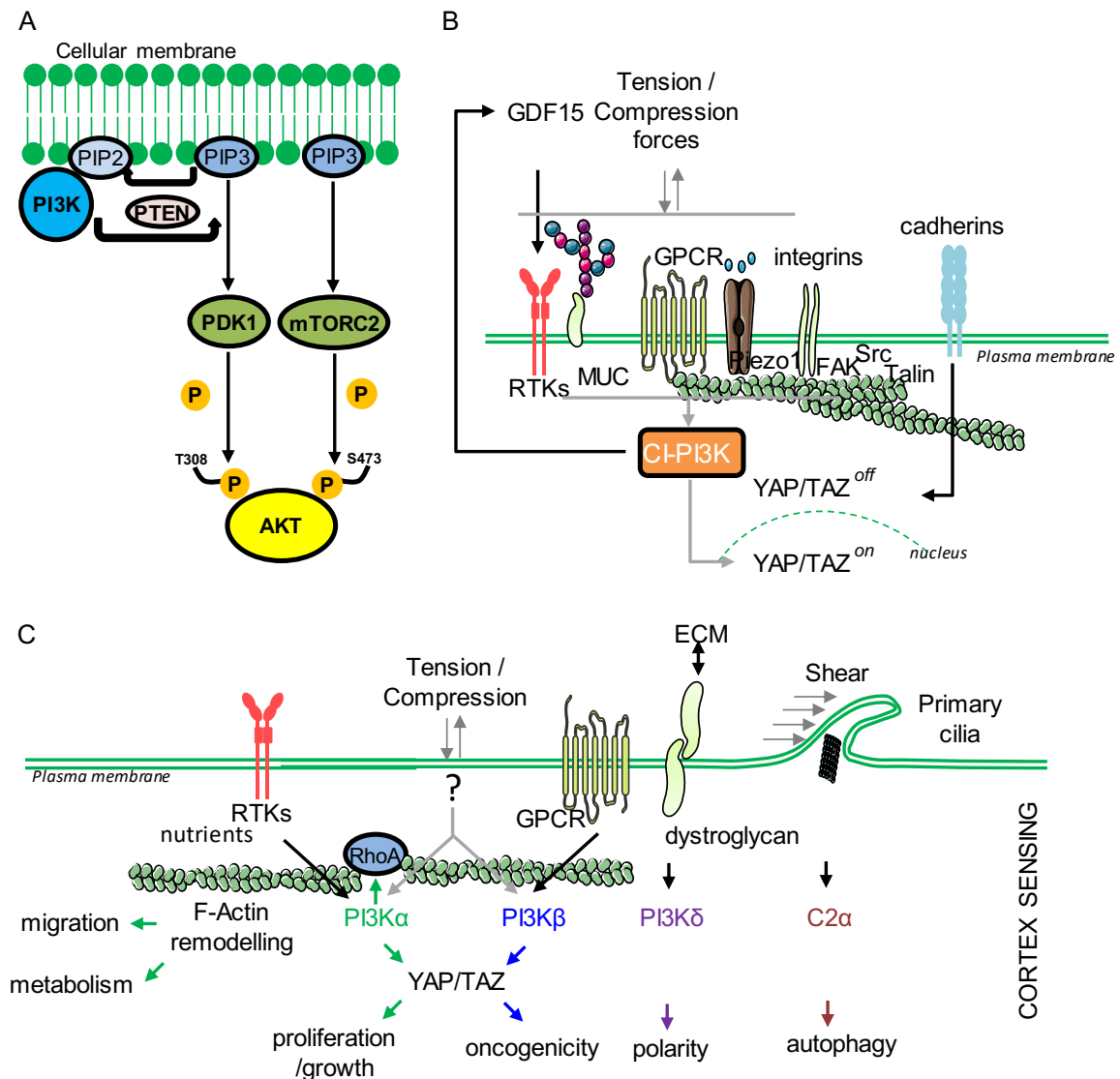
Mechanical parameter of a tissue: During cancer development, changes in mechanical context is observed, including a potential “softening” of tissue prior tumor detection. This mechanical context should be better defined and the type of mechanical cues that a tumor cell encounter (that one could call mechanotype) should be better measured in an aim to control these parameters in *ex vivo* settings.

Figure legends:

Key figure 1:

PI3K signaling, isoform selectivity and mechanosensing at cellular cortex. A. Canonical phosphatidylinositol-3-kinase-AKT (PI3K-AKT) signaling pathway. Class I PI3Ks phosphorylates and transforms phosphatidylinositol-biphosphate (PIP2) into phosphatidylinositol-triphosphate (PIP3). PIP3 induces AKT phosphorylation via PDK1 and mTORC2, promoting mTORC1 activation. **B.** Mechanical constraints couple to cellular processes at plasma membrane through two major molecular events: - translation of cortex membrane tension on actin cytoskeleton, - coupling of tension with intermediate filaments and microtubules that impact nuclear morphology. Compression, tension, and shear stress, converge to modulate cortex tension, referred as cortex sensing. Action on membrane tension activates class I PI3Ks *via* Piezo1 [60], MUC5AC/EGFR [61], MUC13 [42], cadherins [51], integrins and transmembrane proteins Syndecan-4, Focal adhesions proteins such as FAK Src and Talin [35] as well as controls inside-outside signaling [21]. Mechanics-activated class I PI3K promotes secretion of growth factors that feedforward the signal. Recent evidence shows that class I PI3Ks (CI-PI3K) signal also controls the YAP/TAZ mechanosensor switch in order to regulate mechanosensitive gene expression. **C.** Know isoform selective signaling. Under mechanical stress, PI3K α is a major activator of RhoA activity, actin cytoskeleton remodeling and metabolism control, favoring cell migration. RTKs activates PI3K α ; GPCR activates PI3K β . While PI3K α /YAP/TAZ axis is under control of nutrient feeding, only PI3K β /YAP/TAZ axis is fully oncogenic. Dystroglycan through binding with ECM organises cell polarity; recruited PI3K δ critically control epithelial cell polarity. Shear stress is sensed by class II PI3K (C2-PI3K) PI3K $C2\alpha$ in primary cilia, where they are specifically located, and is coupled to autophagy. Only molecular links with mechanical stress that are demonstrated are shown.

Figure 1



Boxes:

Box 1 Shear / tensile / compressive stresses

Cells are subjected to external mechanical stimulation, that can highly modify their behaviour. 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/m², or pascal, like a pressure): the applied force is divided by the cross-sectional area that supports the load. Stress must not be confounded with the strain ϵ , that is the measure of the relative deformation of 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 pressure.

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” 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 higher 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 toward the part on which it acts, it is called “compressive stress” (Figure I-right). As cells grow and divide in a limited space, they push against their surrounding environment to accommodate space for new biomass. Cells act upon the extracellular matrix, adhesive and cohesive bonds, and against steric constraints due to confinement, resulting in growth-induced compressive mechanical stress.

These different forces are known to be typically of the order of magnitude of the kilopascal [62,63].

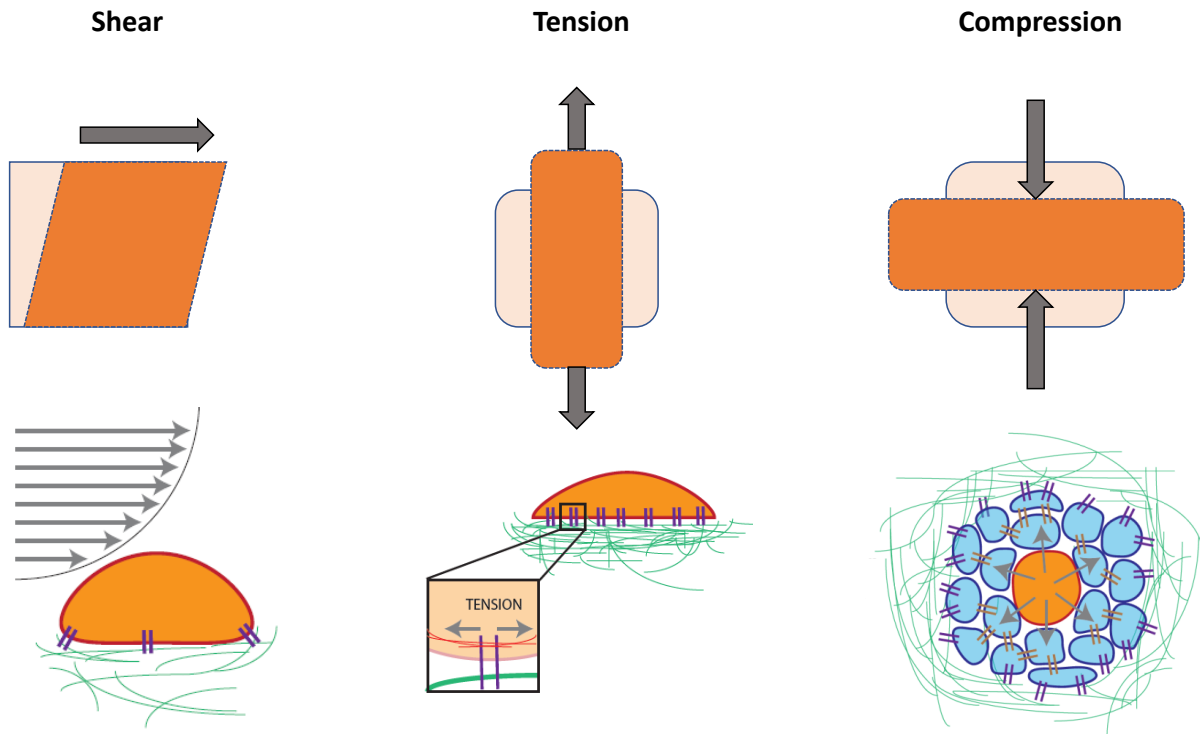


Figure I Legend: Graphical representation of different mechanical stresses applied on cells: shear, tensile and compressive stresses could be applied and participate into mechanical signaling in cells. Orange/blue spheres: cells, green lines: extracellular matrix as collagen fibres, grey arrows: applied forces.

Table 1 Innovating systems that model biomechanical cues in single (2D) or multicellular (3D) setting. This table presents an overview of some achievements, requirements, and limitations for 2D and 3D devices (usually commercially available), and 2D and 3D microfluidic devices, that typically require 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.

| | 2D cultures | 3D cultures | 2D microfluidic devices | 3D microfluidic devices |
|--|------------------------------------|------------------------------------|--|--|
| Budget | Low cost | Low cost | High cost | High cost |
| Design and fabrication | - Simple setups - User friendly | - Simple setups - User friendly | - Complex setups - Difficult to use | - Complex setups - Difficult 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 (Kalli <i>et al.</i>, 2018)[21] | Static (Kim <i>et al.</i>, 2017)[64] | Dynamical (Ho <i>et al.</i>, 2018)[65] | Dynamical (Ahn <i>et al.</i>, 2020) [66] |
|---|--|--|--|--|
| 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 (Alessandri <i>et al.</i> , 2013) [67] | Possible (Gao <i>et al.</i> , 2012) [68] | Possible (Sart <i>et al.</i> , 2017) [69] |
| Addition of sensors | Possible | Possible | Possible | Possible |
| Recollection of sample | Possible | Possible | Never achieved | Never achieved |
| Co-culture of cells | Possible | Possible | Possible | Possible |
| Co-culture with the micro environment | Limited in 2D | Possible | Limited in 2D | Possible (Choi <i>et al.</i> , 2015)[70] |

Table 2 Phosphoinositide 3-kinases and mechanical stress

The Phosphoinositide 3-kinases (PI3K) family is involved in main cell functions such as survival, proliferation, growth, migration, differentiation, protein synthesis and vesicular trafficking. In human, the PI3K family is divided into three different classes based on primary structure, regulation, and *in vitro* lipid substrate specificity [28]. The PI3K classes I, II and III phosphorylate the 3'-position hydroxyl of the D-myo-inositol head group to generate specific phosphoinositide forms [28]. *In vitro*, all classes can generate phosphatidylinositol 3-phosphate [PtdIns-3-P, PtdIns(3)P, PI-3-P], class 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] [71]. Elevated class I PI3K signaling is considered as a hallmark of cancer [72]. Role of class II and III PI3K in cancer is not fully elucidated.

In vertebrate only class I PI3Ks subfamily comprises four members. This class functions as heterodimers with one of four catalytic p110 subunits (p110 α , β , δ or γ) and a regulatory subunit (p85 α , p85 β , p55 γ , p101 or p84) (Table II). Class III PI3Ks is the unique PI3K class conserved in all organisms (*D discoideum*, *melanogaster*, *S cerevisiae*).

Class II PI3Ks subfamily comprises three catalytic isoforms (C2 α , C2 β , and C2 γ), but, unlike classes I and III, no regulatory proteins [28] (Table II).

Class III PI3Ks is more similar to class I in structure, as they function as heterodimers of a catalytic (VPS34) and a regulatory (VPS15/p150) subunits. This class is mainly involved in protein and vesicle trafficking [73] (Table III).

PI3K isoforms were firstly considered as redundant. However, while all synthesizing PIPs, each isoform has a particular role; there is also a heterogeneity of expression of all classes of PI3K in each cell type or tumor type [28].

Isoform are transducing mechanical stresses and their specificity of cell activation at plasma membrane, primary cilia, intracellular vesicles, nucleus or of expression in cancer and stromal cells could more precisely be involved in discriminating the three different types of mechanical stress (shear, tensile, compressive stresses) and determining their cellular output. So far, is demonstrated as transducing i-shear stress, class I [22], class II [23–25] (one study excludes class III PI3K as integrating shear stress; however, this was performed in a limited range of model organisms [23]), ii- tensile stress, class I [18] (roles of class II and III was not studied), iii- compressive stress, class I [20,21,51,64] (importance of class III PI3K in response to compression is highly suggested by litterature [51], role of Class II was not studied so far).

| Subunit | Protein (Human) | Gene name (Human) | Gene name (<i>D melanogaster</i>) | Gene name (<i>D Discoideum</i>) | Gene name (<i>S Cerevisiae</i>) |
|------------------|--|-------------------|-------------------------------------|-----------------------------------|-----------------------------------|
| Class I | | | | | |
| Catalytic | p110 α | <i>PIK3CA</i> | <i>dp110(Dmel\Pi3K92E)</i> | <i>DdPIK1</i> | |
| | p110 β | <i>PIK3CB</i> | | | |
| | p110 δ | <i>PIK3CD</i> | | | |
| | p110 γ | <i>PIK3CG</i> | | | |
| Regulatory | p85 α or p55 α or p50 α | <i>PIK3R1</i> | <i>dp60(Dmel\Pi3K21B)</i> | | |
| | p85 β | <i>PIK3R2</i> | | | |
| | p55 γ | <i>PIK3R3</i> | | | |
| | p101 | <i>PIK3R5</i> | | | |
| | p84 or p87 | <i>PIK3R6</i> | | | |
| | | | | | |
| Class II | | | | | |
| Catalytic | PI3KC2 α | <i>PIK3C2A</i> | <i>Dmel\Pi3K68D</i> | | |
| | PI3KC2 β | <i>PIK3C2B</i> | | | |
| | PI3KC2 γ | <i>PIK3C2G</i> | | | |
| Class III | | | | | |
| Catalytic | Vps34 | <i>PIK3C3</i> | <i>DVps34(Dmel\Pi3K59F)</i> | <i>DdPIK5 (Vps34)</i> | <i>VPS34p</i> |
| Regulatory | Vps15 | <i>PIK3R4</i> | <i>Ird1(Dmel\Vps15)</i> | <i>DdVps15</i> | <i>VPS15p</i> |