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The biomechanical context influences the PI3K output signaling in breast and pancreatic cancer cells.

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Abstract (max 200 words): Mechanical stresses, including compressive stress, are well-recognized as being inherently triggered during cancer development. They are integrated by cells in biochemical signals through mechanotransduction. However, little is known about signaling induced by compressive stress. It arises from extra-cellular matrix remodeling and tumor growth, which is overrepresented in breast and pancreatic tumors. Here, we confirmed transcriptomics data, by experimental data obtained after unidirectional 2D compression of breast and pancreatic cancer cells. Gene expression of PI3K-AKT pathway was enriched under compression in breast and pancreatic cancer cells. Compression induced the overexpression of class I PI3K members and PI3K pathway activation in these two types of cancer cells. When we added pan-PI3K inhibitor and compressive stress alone, cancer cell confluency decreased. Pan-PI3K inhibitor coupled with compression accentuated cell confluency decrease in various breast and pancreatic tumor cells, highlighting the importance of PI3K pathway for cell proliferation under compression. PI3K/AKT signaling is known to closely regulate autophagy process. The level of autophagosome protein GABARAP identified as a key PI3K transcriptional target under compression by our bioinformatics analysis was indeed modulated by compression. In conclusion, targetable mechanisms, such as PI3K signaling or autophagy process, may provide a proliferative advantage and increase cell resistance to compression.

Keywords: PI3K-AKT, class I PI3Ks, compressive stress, mechanotransduction, autophagy, breast and pancreatic cancer cells.

1. Introduction

In tissues, all cells are subjected to mechanical forces, exerting a stress on the cell surface, which can be transmitted notably to the nucleus. Physically, this stress is measured with internal tissue/cell resistance to deformation described in (N/m^2) or Pascal (Pa). Cells encounter 3 types of mechanical stresses: shear, tensile and compressive stress [1]. These mechanical interactions can emerge from cell-cell or cell-substrate interaction [2]. Shear stress occurs when mechanical forces are applied parallel to a surface such as the fluid shear arising from blood flow which physically stresses blood vessel walls. In contrast, tensile and compressive stresses are perpendicularly directed away and toward the cell surface, respectively. In adult physiological condition, most epithelial cells are subjected to limited compression stresses. During solid tumor development, localized tumor

growth in confined space and changes in the stroma lead to the emergence of large compressive stresses. The impact of compressive stress on tumor cell progression and migration depends on the magnitude, duration, and direction of applied forces, and is associated to extra-cellular tissue components [1]. *In vitro,* in most cases, 3D models of solid cancer cells under confinement show a cell proliferation decrease [3], while colon cancer compression without confinement promotes cancer cell growth [4]. Moreover, compression forces can increase cancer cell invasive capabilities as well as their resistance to chemotherapeutic treatments due to a maintained cell survival and reduced proliferation, limiting the effect of both cytotoxic/cytostatic agents [3, 5-7]. Currently, there is no way to predict what will be the cellular physiological response of compression in cancer cells.

Once exerted on a cell, a mechanical stress induces a mechanotransduction response, coupled to modification of gene expression, which is largely associated with Hippo pathway activation under tensile stress [8]. In tumors, Hippo pathway containing transcriptional regulators YAP/TAZ can reprogram cancer cells into cancer stem cells and incite tumor initiation, progression, and metastasis [8]. Further, the Hippo pathway crosstalks with morphogenetic signals, such as Wnt growth factors, and is also regulated by Rho and G protein-coupled receptor (GPCR), cAMP and PKA pathways [9]. These pathways are very well studied individually. In the last decade, research has highlighted the interconnections of signaling pathways, and other key intracellular signals involved in mechanotransduction were noticed [10]. In this context, the importance of phosphoinositide 3-kinases (PI3Ks) in mechanotransduction of cancers is misestimated. Recent evidence shows their implication as a pivotal role in mechanotransduction even if their regulation under compressive stress response is unclear in the literature [10].

PI3K proteins can be divided into three classes (I–III) based on their primary structure, regulation, and *in vitro* lipid substrate specificity [11]. In the literature, the term PI3K refers usually to class I PI3K. This class, composed of four enzymes (α, β, δ, γ), with non-redundant functions [11, 12] and non-redundant roles in cancer [13], generates phosphatidylinositol 3,4,5-trisphosphate (PtdIns-3,4,5-P3 or PIP3) from phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P2 or PIP2) [11]. This reaction is reversed by the phosphatase activity of Phosphatase and TENsin homolog (PTEN). In pancreatic cancer cells, compression-induced PI3K activation promotes migratory phenotype involving an autocrine auto-stimulation loop [6, 7, 14]. Class I PI3Ks are known to be upstream activators of YAP/TAZ transcriptional pathway under tensile stress, positioning class I PI3Ks proteins as potential regulators of an essential mechanotransduction signaling [15]. Further, in breast cancer cells, *in vivo* overexpression of PI3Kβ sensitizes untransformed cells to YAP/TAZ-induced oncogenicity [15]. Moreover, PI3K pathway is one of the most common aberrantly activated pathway in *PIK3CA* mutated breast cancer cells which provided the rationale for development of inhibitors targeting PI3K-AKT pathway (reviewed in [16]).

While the role of tensile stress is now largely recognized as affecting tumor cell fate [17], the role of compressive stress is still emerging. In particular, the global cell signaling modification in response to compression has been poorly characterized. Hence, we decided to use the currently available transcriptomic data obtained after unidirectional compression of breast cancer cells (Kim *et al.*, 2019 [18]) to analyze in an unbiased manner the gene signatures of cell signaling activation including PI3K pathway. Because we found that PI3K activation occurred upon compression, we studied PI3K isoform-specific pathways using known transcriptional targets of PI3Kα inhibitor (BYL719) (Bosch *et al.*, 2015 [19]) or of PI3Kβ inhibitor (AZD8186) (Lynch *et al.*, 2017 [20]). To complete and experimentally demonstrate the relevance of our *in silico* results, we decided to study cell signaling modulation (PI3K, a PI3K-regulated mechanism such as autophagy and YAP/TAZ pathways) under calibrated compressive stress in 2D. We analyzed the same breast cancer cell lines used in Kim *et al.*, 2019 [18] transcriptomic analysis, harboring a constitutive PI3K activation (*PIK3CA*^{E545K}) for MCF-7 cells and KRAS (*KRAS*^{G13N}), TP53 (*TP53*^{R280K}), BRAF (*BRAF*^{G464V}) mutations for MDA-MB-231 cells. Constitutively active mutant RAS proteins bind to and activate PI3K pathways to promote cell-cycle progression. Similarly to breast cancer, pancreatic ductal adenocarcinoma (PDAC) tumors have a significant amount of extra-cellular matrix components (feature called desmoplasia) which amplifies compressive stress felt by cancer cells [6, 18, 21]. Thus, in addition to bioinformatics analysis and breast cancer cells experiments, we added to this study two pancreatic cancer cell lines harboring *KRAS* and *TP53* mutations (PANC-1: *KRAS*^{G12D}, *TP53*^{R273H} and CAPAN-1: *KRAS*^{G12V}, *TP53*^{A159V}); those two mutations are very common in pancreatic cancer[22]. Thus, we analyzed signaling pathway activation in breast and PDAC cancer cells harboring among others a constitutively active PI3Kα, constitutively active KRAS in the context or not of mutant gain of function TP53.

Here, our bioinformatics and experimental data confirmed that PI3K and autophagy pathways merit a greater attention as mechanical sensor of compression. Breast or pancreatic cancers are excellent models to study overexpression and activation of PI3K signaling upon compressive stress.

2. Results

2.1 PI3K-AKT pathway-regulated gene expression is sensitive to compression in breast cancer cells

We first analyzed transcriptomic results in order to understand how compressive stress altered gene signature of cell signaling pathways. For this, we searched for datasets obtained upon compression of cancer cells in Gene Expression Omnibus database (GEO), where public sequencing data from published studies are available (Figure 1A). In GEO database, limited transcriptomic data obtained after cell compression are currently available. Kim *et al*. 2019 [18] compressed breast cancer cells and cancer associated fibroblasts (CAFs) using alginate disks applied on top of 2D cell layers. In this study, Kim *et al*. focused on and analyzed the action of compression on stromal cells. They observed

that mechanical stress in these cells promoted a specific metabolic gene signature increasing glycolysis and lactate production [23]. Here, we investigated the gene expression evolution of two breast cancer cell lines under compression through a bioinformatics analysis. The MDA-MB-231 cell line with a PI3Kα wild-type catalytic domain (*PIK3CA*^{WT}) was compared to MCF-7 containing a constitutive PI3Kα activation (*PIK3CA*^{E545K}) [24]. Both breast cancer cell lines were compressed by a global compression applied on all cells ranging from 0 to 8kPa, and gene expression profile was analyzed. We first searched for gene signature enrichment analysis using canonical signatures from Gene Set Enrichment Analysis (GSEA) (Figure 1). The gene signatures in different pathways were normalized into transcripts per million and compared to housekeeping gene expression such as *ACTINB*, *LAMINA1* and also *LAMIN* gene class. The expressions of these housekeeping genes were not affected by increasing compression (Figure S1A). Under increasing compression [0], [0.3-0.8], [1.5-4.0], [7.0-8.0] kPa, PI3K-AKT signature gene expression was significantly overexpressed in wild-type *PIK3CA* MDA-MB-231 cells, suggesting a global overexpression of genes belonging to these pathways, while it was found already high in constitutively active uncompressed *PIK3CA* MCF-7 cells (Figure S1B, left panel). *PIK3CA*, *PIK3CB* and *PIK3CD* expressions increased in MCF-7 cells and remained stable in MDA-MB-231 cell line (Figure S1B, right panel). However, YAP/TAZ pathway gene signature was not significantly affected by increasing compression in both MCF-7 and MDA-MB-231 cells (Figure S1C, left panel). These transcriptomic results were experimentally confirmed by RT-qPCR analyzing gene expression of YAP/TAZ pathway members such as *TEAD1* and *c-JUN* and of downstream effectors of YAP/TAZ pathway such as *CCN1* and *CCN2* which were not significantly modulated when we applied a 200 Pa/cell 2D compression (200 Pa) using agarose pad with calibrated weight in breast cancer cells (MCF-7 and MDA-MB-231) (Figure S2A) as well as in pancreatic cancer cells (PANC-1 and CAPAN-1) (Figure S2B), except for a modest (1.36-fold) increase expression of *CCN2* in MCF-7 cells (Figure S2A). Further, the ratio p-YAP^{S127}/YAP was not significantly modulated under 200 Pa compression in pancreatic and breast cancer cell lines (Figure S2C).

Taken together, for PI3K family members, these data showed a sensitivity (increased gene expression) to growing under compression (from 0 to 8 kPa) in wild-type *PIK3CA* cells compared to overactivated *PIK3CA* cells.

This sensitivity in signaling pathway expression adds an additional link to search for general PI3K involvement in compressive stress mechanotransduction.

2.2 PI3K pathway-regulated gene expression is sensitive to compression via PI3K α/β isoforms in breast cancer cells

To investigate the specific role of PI3K isoforms, we analyzed data from two more studies which used selective inhibitors of PI3K isoforms: Bosch *et al.*, 2015 [19] and Lynch *et al.*, 2017 [20]. The authors used BYL719 or AZD8186 compounds described as selective inhibitors of PI3Kα or PI3Kβ respectively, in mutant *PIK3CA* MCF-7 and mutant *PTEN* HCC70 breast cancer cells. Mutant PTEN leads to increased PI3K activity [25]. We next used gene expression data from PI3Kα inhibition in MCF-7 and PI3Kβ inhibition in HCC70

cells to define PI3Kα and PI3Kβ signatures. Selective transcriptional targets were crossed-compared with the compression-specific transcriptional targets that we previously identified (Figure 1).

After PI3Kα inhibition in MCF-7, 1279 genes had a significantly altered expression. After PI3Kβ inhibition in HCC70 cells, gene expression of 933 genes was also significantly affected. Gene expression of 1052 targets was affected in compressive conditions ([0.3-0.8], [1.5-4.0] and [7.0-8.0] kPa compared to [0] kPa) in MCF-7 cells. We compared these signatures to the list of the 102 genes found in canonical "PI3K-AKT REACTOME signaling pathway in cancer" signature (Figure 2). PI3Kα and PI3Kβ signatures overlapped with the regulation of 139 genes (listed in Figure 2, top table). Compressive stress gene signature mostly overlapped either with PI3Kα or PI3Kβ signatures, on non-common 32 and 31 genes respectively (listed in Figure 2, left and right tables respectively), suggesting a differential effect on isoform activation in response to compression.

Finally, GABA type A receptor associated protein like 1 gene (*GABARAPL1)*, coding for GABARAP structural protein of autophagosome, was the only common target gene differentially expressed between PI3Kα signature, PI3Kβ signature, compressive stress signature and "PI3K-AKT REACTOME signaling pathway in cancer" signature (Figure 2). *GABARAPL1* was affected under increasing compression in MCF-7 and MDA-MB-231 cells (Figure 2, low panel).

These analyses show that compression and PI3K signaling share common transcriptional targets, and that PI3Kα and PI3Kβ gene signatures are altered upon compression, regardless the mutational status of *PIK3CA* or *PTEN*.

2.3 PI3K pathway still promotes cell confluency under compression in breast and pancreatic cancer cells

To experimentally investigate the involvement of PI3K in mechanotransduction, we treated breast (MCF-7 and MDA-MB-231) and pancreatic (PANC-1 and CAPAN-1) cancer cell lines with a pan-PI3K inhibitor (GDC-0941 (10 μM)) alone or in addition with a 2D compression device for 24h, with a single cell stress evaluated at 200 Pa (200 Pa); we quantified cell confluency after 0, 24h, 48h and 72h. All four cell lines harbored a significant decrease in cell confluency after GDC-0941 or 200 Pa compression compared to uncompressed vehicle control condition (Figure 3A-D). The cell confluency of MCF-7 and CAPAN-1 significantly decreased by combining GDC-0941 and 200 Pa compression compared to GDC-0941 or 200 Pa conditions alone (Figure 2A). Contrariwise, this result was neither found in MDA-MB-231 nor in PANC-1, where 200 Pa alone, GDC-0941 alone or a combination of both showed the same cell confluency values after 72h. This result suggests that these cells were resistant to pan-PI3K inhibitor under 2D compression (Figure 3B,C).

2.4 Compression activates PI3K pathway gene expression in breast and pancreatic cancer cells

To understand those results, we next compressed breast and pancreatic cells and analyzed gene expression of 6 PI3K-AKT pathway genes including the four class-I PI3K genes (*PIK3CA*, *PIK3CB*, *PIK3CD* and *PIK3CG*), *PTEN* and *EIF4A*. mRNA level analysis revealed

respectively a 2.9-fold and 6.2-fold overexpression of *PIK3CA* and *PIK3CD* genes in MCF-7 cells, and a 3.5 and 2.4-fold increase of *EIF4E* gene expression in MCF-7 and MDA-MB-231 cells respectively (Figure 4A). Moreover, at protein level, compression significantly increased by 3.5-fold p-AKT^{S473}/total AKT ratio in MCF-7 (Figure 4C). In PDAC cancer model, cells harbored a 11.7-fold significant *PIK3CG* overexpression in PANC-1 and a 2.7-fold, 4.9-fold significant overexpression of *PIK3CB* and *PIK3CG* respectively in CAPAN-1 (Figure 4B). Further, *PIK3CB* gene expression was also increased, 8.1-fold (p=0.07) and 2.7-fold (p=0.04) in 200 Pa condition in PANC-1 and CAPAN-1 cells respectively (Figure. 4B). Finally, compression significantly increased by 2.9-fold p-AKT^{S473}/total AKT ratio in CAPAN-1 (Figure 4D).

These experimental results show the mRNA overexpression of important PI3K pathway members, that are class-I PI3K genes (*PIK3CA*, *PIK3CB*, *PIK3CD*, *PIK3CG*) as well as the activation of PI3K pathway as assessed by AKT phosphorylation after 2D compression mostly in mammary (MCF-7) and pancreatic cancer (CAPAN-1) cells.

2.5 Compression influences autophagy gene expression in breast cancer cells

PI3Kα and PI3Kβ are known regulators of autophagy process [26, 27]. When comparing PI3Kα and PI3Kβ signatures, PI3K-AKT REACTOME canonical pathway and compressive stress selective gene expression, only *GABARAPL1* gene was significantly affected and overlapped in these four signatures (Figure 2). Briefly, this gene encodes for a structural protein of autophagosome and autophagolysosome involved in autophagy process (reviewed in [28]). *GABARAPL1* gene expression was significantly upregulated after PI3Kα (x1.90; p-value: $1.83.10^{-2}$) and PI3Kβ (x1.42; p-value: $6.15.10^{-3}$) inhibitions. Under increasing compression from [0] kPa up to [7.0-8.0] kPa, *GABARAPL1* gene expression was overexpressed in wild-type PI3K MDA-MB-231 cells, but not in constitutively PI3K-activated MCF-7 cells (Figure 2, lower panel), suggesting a differential coupling of PI3K signal to *GABARAPL1* expression under compression. Moreover, under increasing compression, the canonical autophagy GSEA pathway gene expression significantly increased in wild-type PI3K MDA-MB-231 cells, and significantly decreased in constitutive PI3K activated MCF-7 cells (Figure S1B, right panel).

2.6 Compression modulates the expression of autophagosome protein structure in breast and pancreatic cancer cells

Autophagy process is proposed as a therapeutic targeting pathway for various tumors [26]. By analyzing transcriptomics data, we decided to analyze autophagy gene as well as *GABARAPL1* gene expressions in compressed cells. In MDA-MB-231 cells, no significant modulation of *ATG12*, *ATG4A*, *ATG14*, *PIK3C3*, *PIK3R4* gene expressions (Figure 5A, right panel), however an increasing trend for *ATG12*, *ATG14*, *PIK3C3*, *PIK3R4* gene expressions in MCF-7 cells, after compression for 24h was observed (Figure 5A, left panel). Contrariwise, and in agreement with transcriptomic data analysis (Figure 2, lower panel), *GABARAPL1* gene expression was significantly increased by 2.1-fold (p=0.01) in MDA-MB-231 (Figure 5A, right panel) and not affected in MCF-7 (p=0.36) (Figure 5A, left panel). In addition to *GABARAPL1* gene expression, the GABARAP protein level was analyzed.

According to gene expression, the 200 Pa compression significantly increased by 5.9-fold GABARAP protein level (p=0.04) in MDA-MB-231 and no difference was measured in MCF-7 compared to control (0 Pa) (Figure 5C). Diverse results were obtained in pancreatic cancer cells. PANC-1 cells harbored an increasing trend for *ATG12*, *ATG14*, *PIK3C3* and *PIK3R4* gene expressions (Figure 5B, left panel), however CAPAN-1 cells showed no significant modulation of *ATG12*, *ATG4A*, *ATG14*, *PIK3C3* and *PIK3R4* gene expressions (Figure 5B, right panel) after 200 Pa for 24h. For *GABARAPL1* gene expression, PANC-1 cells harbored a 3.0-fold *GABARAPL1* gene overexpression after 200 Pa compression for 24h (Figure 5D). GABARAP protein level was significantly decreased (0.47-fold; (p=0.003)) in CAPAN-1 cells and not in PANC-1 cells (Figure 5D). However, the basal level of GABARAP was found higher in PANC-1 cells compared to all other cell lines (not shown). These experimental results confirmed a trend in autophagy gene modulation after 2D compression. *GABARAPL1* gene expression was increased in MDA-MB-231 mammary cells and PANC-1 pancreatic cells, the GABARAP protein was increased in MDA-MB-231 and high in PANC-1 cells. Reversely, GABARAP protein expression was low in MCF-7 and decreased in CAPAN-1 cells.

Figures, Tables and Schemes

Figure 1

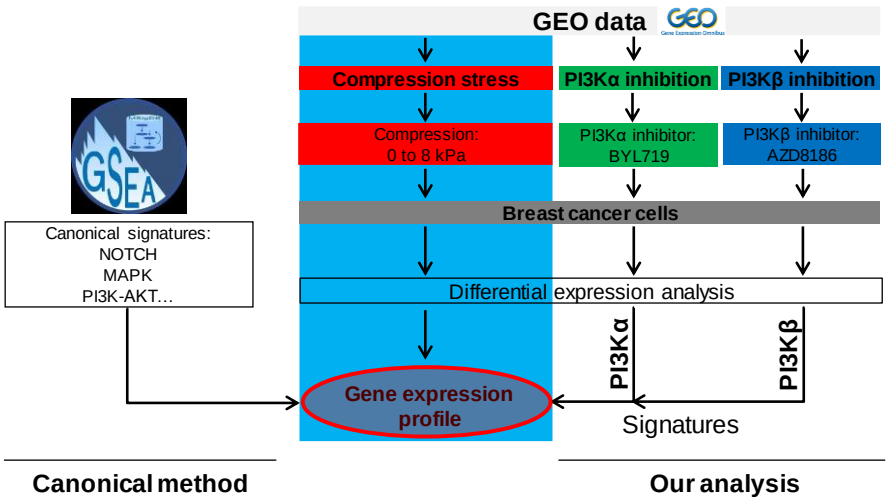

Figure 1. PI3K-AKT: a pathway involved in mechanotransduction in breast cancer cells. Available transcriptomic data from compressed breast cancer cells were downloaded from Gene Expression Omnibus data (GEO) from Kim *et al.*, 2019: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133134. Breast cancer cells (MDA-MB-231: *PIK3CA*^{wt} and MCF-7: (*PIK3CA*^{E545K} mutated) were compressed from 0 to 8 kPa and then a differential expression analysis was performed between 0; [0.3 to 0.8 kPa]; [1.5 to 4.0 kPa]; [7.0 to 8.0 kPa] respectively. On the left side, the canonical analysis method consisting in Gene Set Enrichment Analysis (GSEA) using commonly used canonical signatures. In parallel, differential expression analysis was performed from breast cancer cells treated with two PI3K inhibitors (PI3Kα (BYL719)

and β (AZD8186)) (Bosch *et al.*, 2015 and Lynch *et al.*, 2017). The gene expression profiles after PI3Kα and β inhibitions (PI3Kα and β signatures) were compared to that obtained in the compressive stress study.

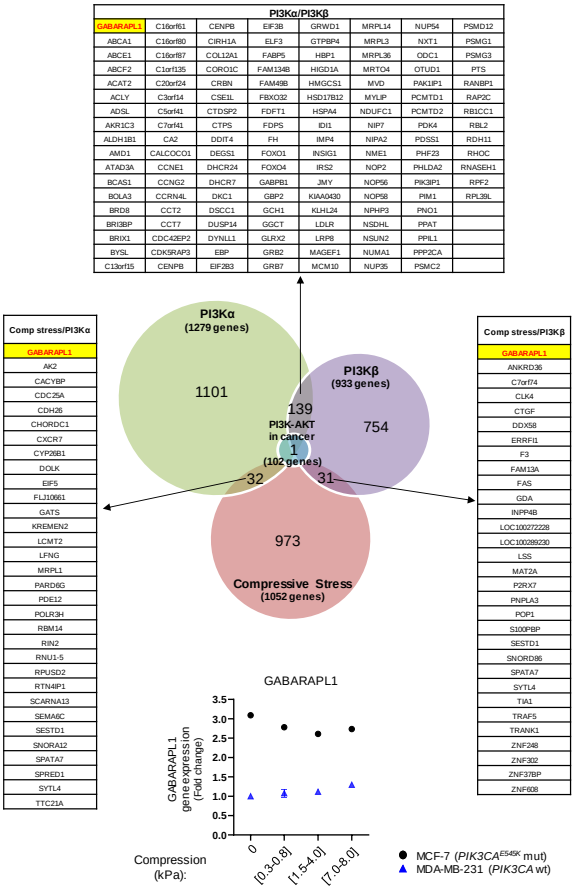

Figure 2. Comparative analysis between compressive stress, PI3Kα/PI3Kβ and PI3K-AKT signaling in cancer gene signatures. The lists of 1279, 933 and 1052 differentially expressed genes in PI3Kα signature, PI3Kβ signature, compressive stress signature and 102 genes of REACTOME PI3K-AKT signaling in cancer signature were compared. Venn diagram shows the overlapping between PI3Kα signature, PI3Kβ signature, compressive stress and REACTOME PI3K-AKT signaling in cancer. Tables represent the overlapping between compressive stress/PI3Kα signature (32 genes) (bottom left), compressive stress/PI3Kβ signature (31 genes) (bottom right) and PI3Kα/PI3Kβ signatures (139 genes) (above center). The only common gene differentially expressed in PI3Kα inhibition/PI3Kβinhibition/compressive stress was *GABARAPL1* (highlighted in yellow). MDA-MB-231 (*PIK3CA*^{wt}; blue triangles) and MCF-7 (PIK3CA^{E545K} mutated, black dots) breast cancer cells were gradually compressed (0 kPa; [0.3 to 0.8 kPa]; [1.5 to 4.0 kPa]; [7.0 to 8.0 kPa] respectively) and *GABARAPL1* gene expression was quantified using Agilent microarray (Data available in https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133134).

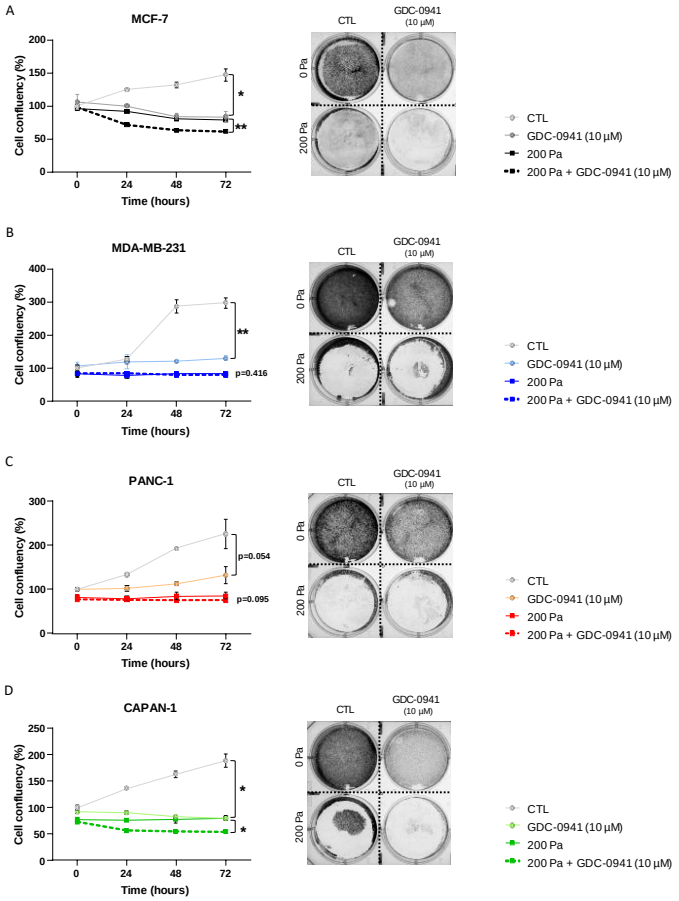

Figure 3. Cell confluency in MCF-7/MDA-MB-231 breast and PANC-1/CAPAN-1 pancreatic cancer cells after 200 Pa compression. After indicated cell treatment and/or compression, cell confluency was quantified using Crystal Violet staining (**A-D**, left). Pictures of representative experiments performed in 6-well plates after 72h are shown (**A-D**, right). **A**. Cell confluency was analyzed at 0, 24, 48 and 72 hours in MCF-7, MDA-MB-231, PANC-1 and CAPAN-1 cells were treated with pan-PI3K inhibitor (GDC-0941 (10µM)) (gray, light blue, orange and light green curves,), 200 Pa compressed (black, dark blue, red and dark green curves) or with pan-PI3K inhibitor (GDC-0941 (10µM)) and 200 Pa compressed (dotted curves) compared to control cells (light gray curves). Quantitative analyses were performed using ImageJ software. Results are presented as mean, +/- SEM, n=3. p=p-value; *p-value<0.05; **p-value<0.01.

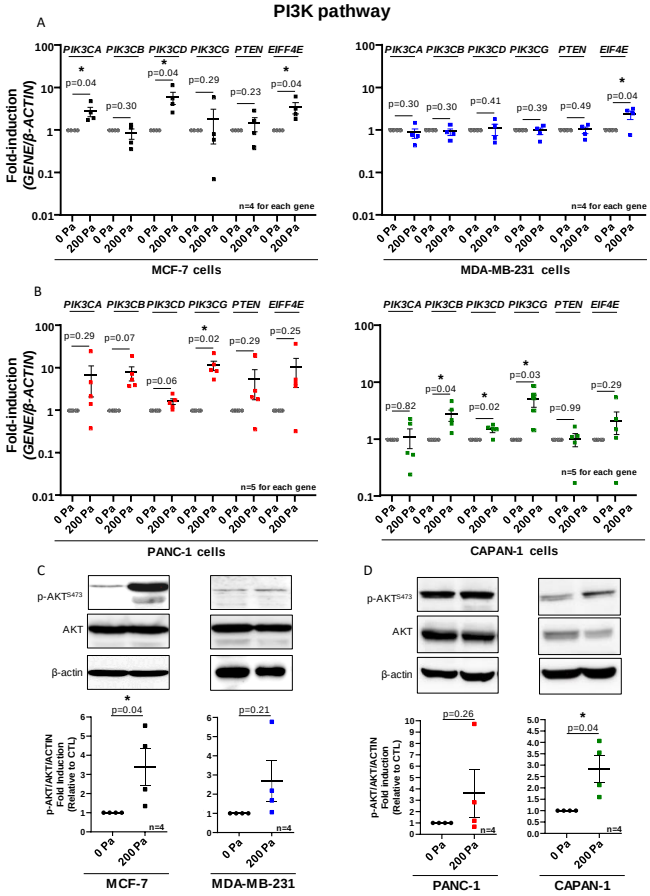

Figure 4. PI3K signaling in breast and pancreatic cancer cells after 200 Pa compression.

A,B. Representation of *PIK3CA*, *PIK3CB*, *PIK3CD*, *PIK3CG*, *PTEN* and *EIF4E* mRNA expressions in MCF-7 (A, left panel), MDA-MB-231 (A, right panel), PANC-1 (B, left panel) and CAPAN-1 (B, right panel) cells compressed at 200 Pa/cell range value in 2D (200 Pa) compared to non-compressed cells (0 Pa) for 24h. β-actin was used as housekeeping gene. Results are presented as mean, +/- SEM. n≥4 for each gene. **C,D**. Representative western blots of p-AKT^{S473} and AKT in MCF-7/MDA-MB-231 and in PANC-1/CAPAN-1 cells under 200 Pa compression (200 Pa) and non-compressed cells (0 Pa) for 24h. β-ACTIN was used as loading control. p-AKT^{S473}/AKT quantitative analyses were performed using ImageJ software. Results are presented as mean, +/- SEM, n=4. *p-value<0.05.

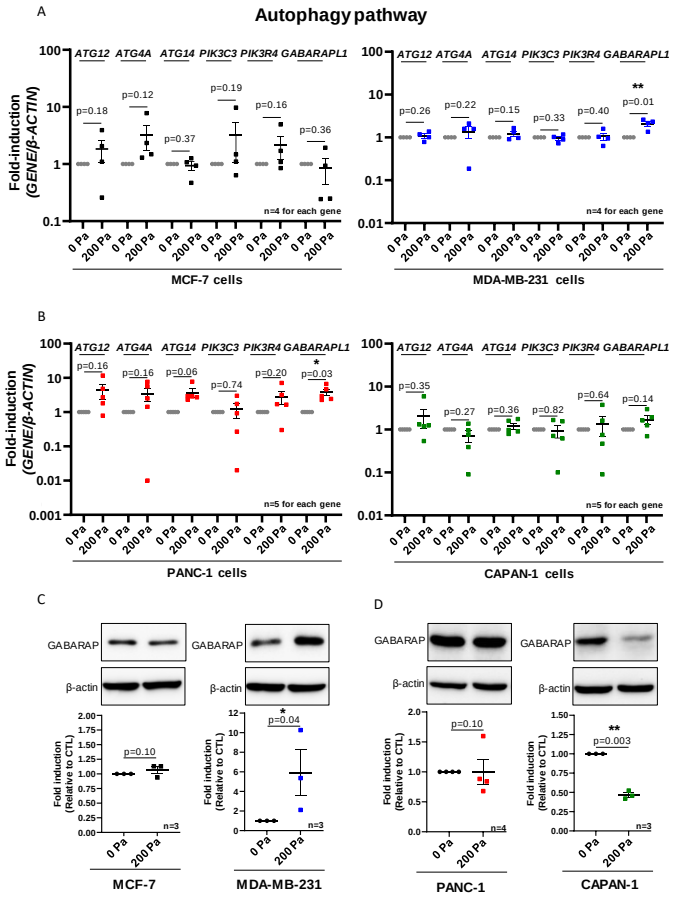

Figure 5. Autophagy pathway in breast and pancreatic cancer cells after 200 Pa compression. **A,B**. Representation of *ATG12*, *ATG4A*, *ATG14*, *PIK3C3*, *PIK3R4* and *GABARAPL1* mRNA expressions in MCF-7 (A, left panel) and MDA-MB-231 (A, right panel) and in PANC-1 (B, left panel) and CAPAN-1 (B, right panel) cells 200 Pa compressed (200 Pa) compared to non-compressed cells (0 Pa) for 24h. β-actin was used as housekeeping gene. Results are presented as mean, +/- SEM. n≥4 for each gene. **C,D**. Representative western blots of GABARAP in MCF-7 and MDA-MB-231 (C) and in PANC-1 and CAPAN-1 cells (D), 200 Pa compressed (200 Pa) and non-compressed cells (0 Pa) for 24h. 20 µg of protein were loaded in each lane. β-ACTIN was used as loading control. GABARAP/β-ACTIN quantitative analyses were performed using ImageJ software. Results are presented as mean, +/- SEM, n≥3. *p-value<0.05; **p-value<0.01.

3. Discussion

The PI3K pathway is essential for cell proliferation, survival, and metabolism [29, 30]. Many studies describe the cellular effects of PI3Kα/β inhibition in different cancer models [12]. However, limited high throughput RNA sequencing (RNAseq) data are available after PI3K inhibition and a similar lack of transcriptomic data in cells subjected to compressive stress exists. Here, we provide RNAseq data analysis and experimental evidence that the targetable PI3K signaling is sensitive to compressive mechanical forces. This knowledge is important to develop efficient targeted therapeutics in breast and pancreatic cancer.

In human cancers, the *PIK3CA* gene is frequently mutated mostly in hot spot regions. The hotspot E542K and E545K mutations that reside in the p110α helical domain are found in over a third of estrogen receptor (ER)-positive breast cancer, representing the most common genomic alteration in this group of tumors [31]. Our study focuses on two breast cancer cell lines MCF-7 containing a constitutive PI3Kα activation (*PIK3CA*^{E545K}), and MDA-MB-231 harboring a wild-type *PIK3CA* and containing a high level of PTEN and low level of phosphorylated AKT [32]. PTEN phosphatase possesses a PI3K antagonist action balancing the activation of the canonical AKT-mTORC1 pathway [33]. Therefore, comparing MCF-7 and MDA-MB-231 allows to compare two cell lines harboring different *PIK3CA* mutational profile and similar AKT phosphorylation on serine 473 (Figure 4C). We highlight as a consequence that compression increase PI3K-AKT-mTORC1 axis in MCF-7 sensitive cell line. We found that compressive stress affects class I *PIK3* gene expressions, significantly for *PIK3CA*, *PIK3CD* and *EIF4E* gene expressions in constitutively active PI3Kα MCF-7 cells. Moreover, a 2D compression significantly increased the p-AKT^{S473}/total AKT ratio in MCF-7 but not in wild-type *PIK3CA* MDA-MB-231 cells and with no other modulation of class I *PIK3* genes expression in MDA-MB-231 cell line. In parallel, in CAPAN-1 cells, we noticed a significant overexpression of *PIK3CB* and *PIK3CG* genes, associated with an increase of p-AKT^{S473}/total AKT ratio compared to PANC-1 cells which harbored no significant modulation of PI3K pathway, excepted *PIK3CG* gene overexpression after 2D compression. These data can be associated with the sensitivity or resistance to GDC-0941 treatment in MCF-7/CAPAN-1 or in MDA-MB-231/PANC-1 under 2D compression.

In molecular terms, MCF-7 and CAPAN-1 sensitive cells share a possible dependency to PI3Kα activity, explained by constitutively active *PIK3CA*^{E545K} mutation for MCF-7 cells and *KRAS*^{G12V} for CAPAN-1 cells. Evidence in the literature show that mutant RAS proteins bind to RAF/MAPK complex and to class I PI3K [34]. Once GTP was bound on RAS protein, RAS regions harbor significant structural changes and increase the RAS affinity for its targets [35]. The direct interaction between RAS-GTP and the PI3Kα p110 subunit (p110α) increases the p110α activity and induces conformational changes stimulating its catalytic activity [36]. Indirectly, RAS acts to stabilize the class I PI3K at the plasma membrane and the PI3K activation was mediated by the receptor tyrosine kinase (RTK) [37].

Further, during cell cycle, PI3K is one of the principal effectors of RAS in the regulation of G0 to G1 transition as well as entrance into S phase [38]. Thus, by stimulating proliferation and further [39] preventing apoptosis, RAS–PI3K signaling supports tumor growth. However, the CAPAN-1, MDA-MB-231 and PANC-1 cells share a KRAS mutation (KRAS^{G12V} in CAPAN-1, KRAS^{G13D} in MDA-MD-231 and KRAS^{G12D} in PANC-1 [40-42]). Mutations in the same gene of the RAS family, including KRAS, cannot be conceived as similar as they differently affect the clinical outcome of the disease and response to chemotherapy; it is in particular relevant for the coupling to PI3K isoforms [34, 43].

CAPAN-1, MDA-MB-231 and PANC-1 harbors a p53 mutation (p53^{A159V} in CAPAN-1, p53^{R280K} in MDA-MD-231 and p53^{R273H} in PANC-1 [44-46]). In physiological conditions, p53

regulates the transcription of four genes, PTEN, IGF-BP3, TSC2, and AMPK in the PI3K-AKT-mTOR pathway, which then all negatively regulate AKT-kinase activity and mTOR, leading to a decrease in cell growth and a reversal of the cancer phenotype. In cancer conditions, the dysregulation of p53 releases the negative regulation of PTEN on PI3K-AKT-mTOR pathway and thus contribute to the overactivation of AKT and cell proliferation associated to tumor progression and growth [47].

Further, MDA-MB-231 and PANC-1 share a CDKN2A gene deletion [40, 48]. If combination of PI3K inhibitors and CDK 4/6 Inhibitors sensitize *PIK3CA* mutant breast cancer cells to PI3K inhibitors [49], this deletion might provide a proliferation advantage under compression, independently of PI3K activity, in the two cell lines that become resistant to PI3K inhibition.

Finally, another cell process, closely regulated by PI3K activity is the autophagic flux, participating to the cell homeostasis and known as a key pathway in therapeutic strategy for a variety of tumors. By comparing PI3Kα/PI3Kβ inhibitions, compressive stress-regulated genes and reactome PI3K-AKT signaling in cancer pathway lists of genes, only *GABARAPL1* gene was significantly affected and overlap in these four studies. Moreover, the GABARAP protein was modulated by compression in breast and pancreatic cancer cells, and GABARAP protein level increased or remained high in the two cell lines that become resistant to PI3K inhibition under compression. GABARAP protein is cleaved by ATG4B protease before its conjugation to phospholipids. This modified lipidated form is localized on the surface of autophagosomes and lysosomes, participating in their formation and promote tubulin polymerization and bundling to form microtubules and therefore involved in autophagy process (reviewed in [28]). Autophagy is a highly conserved catabolic process, participating to the balance of cell proliferation/death in tumor and the tumor microenvironment regulation. This process is highly regulated by PI3K-AKT-mTOR pathway in various tumors. We know that inhibition of PI3K-AKT-mTOR induces autophagy as part of a tumor growth suppressor in early cancerogenesis [50, 51]. The differential expression of GABARAP protein raises the possibility of a modulation of autophagy flux (acceleration or blocking) and certainly of the stability of autophagosomes under compressive stress. Further, we know that induction of autophagy is associated with mechanical stress [26, 52]. In *Dictyostelium discoideum*, an organism which does not present any homolog of vertebrate Class II PI3K, compression activates autophagy in a mTORC1 independent manner [53]. This mechanism, if confirmed in mammalian cells, could participate in cancer cell survival as well as resistance to chemical treatments under compression.

Under increasing compression, gene/protein expression or phosphorylation status of PI3K-AKT and autophagy pathway members were significantly modulated. However, and surprisingly, "YAP/TAZ *via* Hippo" or "YAP/TAZ Non Hippo" pathway gene signatures, known as key regulator of mechanotransduction under tensile stress [54], were not significantly affected in breast and pancreatic cancer cells under unidirectional 2D compressive stress (Figure S1B). In compressive stress context, the implication of YAP/TAZ pathway was less investigated. It was assumed that this stress would induce the

same signal pathways than tensile stress. This assumption could have been prompted by the fact that some techniques used to induce compression could also promote tensile stress [55, 56]. In our study or Kim *et al*. 2019 study [18], cells were compressed in 2D using agarose pad or alginate disk without implementing additional tensile stress. We could argue that a threshold of tensile stress may be needed to permit the activation of YAP/TAZ. If our data seem to confirm those findings in the context of unidirectional compressive stress, differential cytoskeleton remodeling cannot be completely ruled out in our experimental conditions. The exact type of cytoskeleton remodeling under each mechanical stress may also be key to understand cell mechanotransduction processes, as cells adapt to the environmental stresses. Finally, PI3K signal is permissive for tensile stress control of YAP/TAZ and targeting of PI3K could be a novel strategy to hinder the potential YAP/TAZ oncogenic dependence [10].

Altogether, we show that breast and pancreatic cancers are sensitive to compression [6]. Further, ECM and desmoplasia may even be heterogeneously distributed in each PDAC tumor and influence environment rigidity during progressive stages of the disease [21]. In addition to ECM-induced compressive stress, ECM also influences the tumor progression *via* epithelial-mesenchymal transition (EMT) and further contributes to resistance to chemotherapeutic agents [57]. Even if these high compressive stress situations occur mainly in breast cancer and PDAC [14, 58], our study provides an exploratory proof-of-concept of the impact of mechanical stresses on targetable oncogenic cell signaling. The interaction between signal targeted therapies and mechanotherapeutics should be investigated in more solid cancers.

Others have trialed therapeutic strategies that rely on decompression of solid tumor by reducing surrounding fibrosis and facilitating the accession of chemotherapeutic agents to the tumor [59-61]. They used monoclonal antibodies targeting either connective tissue growth factor (CTGF) (pamrevlumab, phase III), or extra-cellular matrix integrins (abituzumab, phase II), or the FAKs (defactinib or GSK2256098, phase II) for pancreatic tumors or tumor growth factor β (LY3200882, phase I) in the case of certain solid tumors such as hepatocarcinomas [59]. These treatments reduce the surrounding fibrous tissue around the tumor and therefore decrease compression forces induced by fibrosis. Some of these trials were stopped because they could accelerate the disease [61]. As compressive forces decrease proliferation of most cancer cells in *in vitro* 3D models, by reducing the surrounding fibrosis, those therapeutics could allow to increase the cancer cell proliferation and promote tissue invasion and disease progression. For these reasons, we are convinced that the balance between therapies reducing fibrosis and fibrosis-induced compression should be well mastered and individually adapted. Our findings also demonstrate that those mechanotherapeutics (modulating the mechanical environment in tumors) could also be used as a way to sensitize to oncogene targeted therapies [61].

4. Conclusion

In addition to the canonical pathways (YAP/TAZ and Piezo) associated with mechanical stresses, our study provides evidence for the role of PI3K-AKT pathway in compression-

induced mechanotransduction, through class I PI3Ks. Moreover, other mechanisms inherent to compressive stress, such as autophagy induction, may provide a proliferative advantage and sustain cell survival in compressive stress environment. Finally, oncogenic alterations are known to skew normal cell signaling. Associated to genetic alterations, environmental context such as compressive stress could also activate oncogenic pathways and induce novel tumoral vulnerabilities independently from their mutational status.

5. Materials and Methods

Transcriptomic analysis

Differential expression analysis after compressive stress in MCF-7 and MDA-MB-231 cells were performed from data provided by Kim *et al.*, 2019 [18]. MCF-7 and MDA-MB-231 breast cancer cells were gradually compressed (0; 0.3 to 0.8; 1.5 to 4.0; 7.0 to 8.0 kPa) and gene expression was quantified using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381, data available at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133134. The two PI3Kα and PI3Kβ inhibition signatures were performed from differential expression analysis after PI3Kα inhibition with BYL-719 (1 μM for 16 to 48 hours) in MCF-7 breast cancer cells (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64033) [19] or PI3Kβ inhibition after AZD8186 treatment (100mg/kg twice daily for 5 days) in HCC70 cells (https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-4656/) [20]. For compressive stress and PI3Kα and PI3Kβ inhibition signatures, differential expression analysis was performed using DESeq2 v1.26.0 [62] on R version 3.6.3 with adjusted p-value <0.05. All differential expression data are relative data from normalized transcript per million (TPM). P-values on graphs were calculated using t-test comparing no compression condition (0 kPa) to each compression condition ([0.3-0.8], [1.5-4.0] or [7.0-8.0] kPa). Enrichment analysis were performed using Gene Set Enrichment Analysis software (GSEA), p-value <0.05 and Fold Discovery Rate (FDR)<25%.

Cell culture

MCF-7 (ATCC; #CRL-3435), MDA-MB-231 (ATCC; # CRM-HTB-26), PANC-1 (ATCC; #CRL-1469) and CAPAN-1 cells (ATCC; #HTB-79) were cultured in Dulbecco's Modified Eagle Medium (GIBCO; #61965026) supplemented with 10% fetal bovine serum (Eurobio Scientific; #CVFSVF00-01), Penicillin-Streptomycin 50 U/mL and 50 μg/mL respectively (Sigma-Aldrich; #P0781), 2 mM L-Glutamine (Sigma-Aldrich; #G7513) and Plasmocin 25 μg/mL (InvivoGen; #ant-mpp) and maintained at 37°C in humidified atmosphere with 5% CO2. Cells were tested for their absence of mycoplasma infection prior use.

2D compression

$2*10^5$ MCF-7, MDA-MB-231, PANC-1 or CAPAN-1 cells were plated in a 35 mm petri dish. MCF-7 and MDA-MB-231 were plated using Dulbecco's Modified Eagle Medium (GIBCO; #61965026) supplemented with indicated products in previous paragraph, however without phenol red. 24h after plating, a 2% low gelling temperature agarose pad (Sigma-Aldrich; #A0701-25G) in Phosphate Buffered Saline (PBS) containing 0.5 mM $MgCl_2$ and 0.9 mM $CaCl_2$ respectively (Sigma-Aldrich; #D8662) was deposited on cells. 2D compression was calibrated at 200 Pa per cell adjusting the weight with small metal plates on 2% agarose pad and calculated using the formula: P=(1/CN)*(1/CS)*m*g (with P=Pressure in Pa; CN=Cell number; CS=Cell surface in m^2; m=mass of the pad and metal plate in kg; g=gravitational force=9.80665 m/s-2). Controls were performed adding the volume of PBS 0.5 mM $MgCl_2$ and 0.9 mM $CaCl_2$ equivalent to the volume of the agarose pad. After 24h of compression, cells were harvested, and RNA/proteins were extracted.

RNA extraction

Cells were rinsed twice with PBS. Cell lysis was performed scraping cells in 1 volume of TRIzol reagent (Ambion; #15596018), and incubating 5min on ice. Then, 1/5 volume of chloroform (Sigma Aldrich; #32211) was added to the mix Trizol/cell lysate. After 3min incubation at room temperature, cell lysate was centrifuged at 12,000g and 4°C, for 10min and the supernatant was collected. RNA was precipitated by adding 1 volume of isopropanol (Sigma Aldrich; #33539), incubated for 10min at room temperature and then centrifuged at 12,000g and 4°C for 10min. The RNA pellet was rinsed using 70° ethanol. After a 5min centrifugation at 7,500g and 4°C the supernatant was removed, the RNA pellet was air dried for 5min and resuspended in 30µL of RNAse free water. RNA concentration was measured using NanoDrop™ 2000 (ThermoFischer scientific).

Reverse transcription and quantitative PCR

cDNA synthesis was performed with iScript kit (BioRad; #1708891) according to the manufacturer protocol using C1000 Touch Thermal Cycler (BioRad) and the following conditions: annealing: 5min at 25°C, reverse transcription: 20min at 46°C, reverse transcriptase inactivation: 1min at 95°C. cDNAs were diluted to a half in RNAse free water. Gene expression was quantified with the SsoFast EvaGreen supermix kit (BioRad, #1725204) using a thermocycler (StepOne™ #4376374; Software v2.2.2) with the following conditions: 20sec at 95°C, 40 denaturation cycles: 3sec at 95°C, annealing and elongation: 30sec at 60°C. β-actin was used as housekeeping gene. The primers used are described in Supplementary Table 1 and each amplicon was validated by sequencing. Gene expression quantification was performed using the Livak method: 2-(Delta Delta C(T)) [63]. Distinct RNA samples ≥4 were analyzed, each amplification was performed in technical duplicate.

Protein extraction

Cells were rinsed with PBS and detached by scrapping in PBS. Cells were harvested through a 5min centrifugation at 3,000g and 4°C. Cells were resuspended in a lysis buffer containing 150 mM NaCl, 50 mM Tris, 1 mM EDTA, 1% Triton, 2 mM dithiothreitol, 2 mM Sodium fluoride, 4 mM Sodium Orthovanadate and supplemented with protease inhibitors (Complete protease inhibitors, Roche). After a 20min incubation on ice, a 10min centrifugation was performed at 12,000g and 4°C and the supernatant was collected. Protein concentration was measured using Bicinchoninic assay (BC Assay Protein Quantification Kit, #3082, Interchim).

Western Blot

20 µg of proteins were separated on a 10% polyacrylamide gel and transferred on nitrocellulose membranes (Amersham™ Protran®; #106000004) using Trans-Blot® Turbo™ (BioRad). Blocking was performed through a 1h incubation in 5% milk. Membranes were incubated overnight at 4°C with primary antibodies in TBS-Tween 0.1%, 5% Bovine Serum Albumin as indicated in Supplementary Table 2. Then, membranes were rinsed three times with Tris-buffered saline 0,1% Tween (TBS-Tween 0.1%), incubated for 1h with secondary antibodies coupled to a horseradish peroxidase, in 1% low fat milk described in Supplementary Table 2. Membranes were next washed three times (1min, 5min and 10min) with TBS-Tween 0.1%. Proteins were detected through chemiluminescence (Clarity™ Western ECL Substrate; BioRad; #1705061) using Chemidoc™ Imaging System (BioRad) and quantified using ImageJ software. ≥3 independent replicates were performed for each protein analyzed.

Cell confluency assay

Cells were compressed following the protocol described above or /and treated with 10µM GDC-0941 (pan-PI3K inhibitor; Axon Medchem) for 0h, 24h, 48h or 72h. After compression ± pan-PI3K inhibitor, cells were rinsed with PBS (Eurobio Scientific, #CS1PBS 0101) and fixed for 15min using PBS containing 10% methanol and 10% acetic acid. Cells were stained for 15min using crystal violet (Sigma Aldrich; #HT90132). Images were performed using Chemidoc™ Imaging System (BioRad) and quantified using ImageJ software.

Statistical analysis

Comparisons between two experimental groups were performed using a paired two-tailed Student t-test, p-value<0.05 was considered significant. All analysis were performed using GraphPadPrism 9.3.1 software.

Figure S1. Housekeeping genes, PI3K-AKT, YAP/TAZ and autophagy pathways under increasing compressive stress in breast cancer cells. MDA-MB-231 (PIK3CAwt; blue squares) and MCF-7 (PIK3CA^{E545K} mutated, black squares) breast cancer cells were gradually compressed (0 kPa; [0.3 to 0.8 kPa]; [1.5 to 4.0 kPa]; [7.0 to 8.0 kPa] respectively) and gene expression was quantified using Agilent microarray (Data available in https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133134). Differentially expressed genes (p-value<0.05) were correlated. **A.** ACTINB (1 gene) and LAMINA1 (1 gene) and LAMIN gene class (12 genes) as housekeeping genes. **B.** Regulation of PI3K-AKT pathways and differential expression of class I PI3K members under increasing compression. **C.** Regulation of mechanoregulation and pathology of YAP/TAZ via HIPPO and non HIPPO mechanisms (45 genes) and autophagy (35 genes) pathways. ± SEM. *<0.05; **<0.01; ***<0.001.

Figure S2. YAP/TAZ pathway under 200 Pa compressive stress in breast and pancreatic cancer cells. A) MCF-7 and MDA-MB-231 breast cancer cells were compressed at 200 Pa for 24h and TEAD1, c-JUN, CCN1 and CCN2 mRNA expressions were quantified. β-ACTIN was used as housekeeping gene. B) PANC-1 and CAPAN-1 pancreatic cancer cells were 200 Pa compressed for 24h and TEAD1, c-JUN, CCN1 and CCN2 mRNA expressions were quantified. β-ACTIN was used as housekeeping gene. C) MCF-7 and MDA-MB-231 breast and PANC-1 and CAPAN-1 pancreatic cancer cells were 200 Pa compressed for 24h and p-YAPS127/YAP protein expressions were quantified. β-ACTIN was used as loading control.

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Data Availability Statement:

For this work, we utilized transcriptomic data from compressed breast cancer cells from Kim *et al.*, 2019 [18], from Gene Expression Omnibus GEO accession number: GSE133134. Our work correlates compression data to PI3Kα inhibition data from Bosch *et al.*, 2015 [19] GEO accession number: GSE64033) and PI3Kβ inhibition data from Lynch *et al.*, 2017 [20] ArrayExpress: ENA-ERP015852).

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References

1. Northcott, J. M.; Dean, I. S.; Mouw, J. K.; Weaver, V. M., Feeling Stress: The Mechanics of Cancer Progression and Aggression. *Front Cell Dev Biol* **2018,** 6, 17.
2. Levental, K. R.; Yu, H.; Kass, L.; Lakins, J. N.; Egeblad, M.; Erler, J. T.; Fong, S. F.; Csiszar, K.; Giaccia, A.; Weninger, W.; Yamauchi, M.; Gasser, D. L.; Weaver, V. M., Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **2009,** 139, (5), 891-906.
3. Delarue, M.; Montel, F.; Vignjevic, D.; Prost, J.; Joanny, J. F.; Cappello, G., Compressive stress inhibits proliferation in tumor spheroids through a volume limitation. *Biophys J* **2014,** 107, (8), 1821-1828.
4. Mary, G.; Malgras, B.; Perez, J. E.; Nagle, I.; Luciani, N.; Pimpie, C.; Asnacios, A.; Pocard, M.; Reffay, M.; Wilhelm, C., Magnetic Compression of Tumor Spheroids Increases Cell Proliferation In Vitro and Cancer Progression In Vivo. *Cancers (Basel)* **2022,** 14, (2).
5. Rizzuti, I. F.; Mascheroni, P.; Arcucci, S.; Ben-Meriem, Z.; Prunet, A.; Barentin, C.; Riviere, C.; Delanoe-Ayari, H.; Hatzikirou, H.; Guillermet-Guibert, J.; Delarue, M., Mechanical Control of Cell Proliferation Increases Resistance to Chemotherapeutic Agents. *Phys Rev Lett* **2020,** 125, (12), 128103.
6. Kalli, M.; Minia, A.; Pliaka, V.; Fotis, C.; Alexopoulos, L. G.; Stylianopoulos, T., Solid stress-induced migration is mediated by GDF15 through Akt pathway activation in pancreatic cancer cells. *Sci Rep* **2019,** 9, (1), 978.
7. Kalli, M.; Li, R.; Mills, G. B.; Stylianopoulos, T.; Zervantonakis, I. K., Mechanical Stress Signaling in Pancreatic Cancer Cells Triggers p38 MAPK- and JNK-Dependent Cytoskeleton Remodeling and Promotes Cell Migration via Rac1/cdc42/Myosin II. *Mol Cancer Res* **2021**.
8. Cobbaut, M.; Karagil, S.; Bruno, L.; Diaz de la Loza, M. D. C.; Mackenzie, F. E.; Stolinski, M.; Elbediwy, A., Dysfunctional Mechanotransduction through the YAP/TAZ/Hippo Pathway as a Feature of Chronic Disease. *Cells* **2020,** 9, (1).
9. Piccolo, S.; Dupont, S.; Cordenonsi, M., The biology of YAP/TAZ: hippo signaling and beyond. *Physiol Rev* **2014,** 94, (4), 1287-312.
10. Di-Luoffo, M.; Ben-Meriem, Z.; Lefebvre, P.; Delarue, M.; Guillermet-Guibert, J., PI3K functions as a hub in mechanotransduction. *Trends Biochem Sci* **2021**.
11. Vanhaesebroeck, B.; Guillermet-Guibert, J.; Graupera, M.; Bilanges, B., The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol* **2010,** 11, (5), 329-41.
12. Arcucci, S.; Ramos-Delgado, F.; Cayron, C.; Therville, N.; Gratacap, M. P.; Basset, C.; Thibault, B.; Guillermet-Guibert, J., Organismal roles for the PI3Kalpha and beta isoforms: their specificity, redundancy or cooperation is context-dependent. *Biochem J* **2021,** 478, (6), 1199-1225.
13. Pons-Tostivint, E.; Thibault, B.; Guillermet-Guibert, J., Targeting PI3K Signaling in Combination Cancer Therapy. *Trends Cancer* **2017,** 3, (6), 454-469.
14. Thibault, B.; Ramos-Delgado, F.; Pons-Tostivint, E.; Therville, N.; Cintas, C.; Arcucci, S.; Cassant-Sourdy, S.; Reyes-Castellanos, G.; Tosolini, M.; Villard, A. V.; Cayron, C.; Baer, R.; Bertrand-Michel, J.; Pagan, D.; Ferreira Da Mota, D.; Yan, H.; Falcomata, C.; Muscari, F.; Bournet, B.; Delord, J. P.; Aksoy, E.; Carrier, A.; Cordelier, P.; Saur, D.; Basset, C.; Guillermet-Guibert, J., Pancreatic cancer intrinsic PI3Kalpha activity accelerates metastasis and rewires macrophage component. *EMBO Mol Med* **2021,** 13, (7), e13502.
15. Zhao, Y.; Montminy, T.; Azad, T.; Lightbody, E.; Hao, Y.; SenGupta, S.; Asselin, E.; Nicol, C.; Yang, X., PI3K Positively Regulates YAP and TAZ in Mammary Tumorigenesis Through Multiple Signaling Pathways. *Mol Cancer Res* **2018,** 16, (6), 1046-1058.
16. Ellis, H.; Ma, C. X., PI3K Inhibitors in Breast Cancer Therapy. *Curr Oncol Rep* **2019,** 21, (12), 110.
17. Stylianopoulos, T., The Solid Mechanics of Cancer and Strategies for Improved Therapy. *J Biomech Eng* **2017,** 139, (2).

18. Kim, B. G.; Sung, J. S.; Jang, Y.; Cha, Y. J.; Kang, S.; Han, H. H.; Lee, J. H.; Cho, N. H., Compression-induced expression of glycolysis genes in CAFs correlates with EMT and angiogenesis gene expression in breast cancer. *Commun Biol* **2019,** 2, 313.
19. Bosch, A.; Li, Z.; Bergamaschi, A.; Ellis, H.; Toska, E.; Prat, A.; Tao, J. J.; Spratt, D. E.; Viola-Villegas, N. T.; Castel, P.; Minuesa, G.; Morse, N.; Rodon, J.; Ibrahim, Y.; Cortes, J.; Perez-Garcia, J.; Galvan, P.; Grueso, J.; Guzman, M.; Katzenellenbogen, J. A.; Kharas, M.; Lewis, J. S.; Dickler, M.; Serra, V.; Rosen, N.; Chandarlapaty, S.; Scaltriti, M.; Baselga, J., PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor-positive breast cancer. *Sci Transl Med* **2015,** 7, (283), 283ra51.
20. Lynch, J. T.; Polanska, U. M.; Delpuech, O.; Hancox, U.; Trinidad, A. G.; Michopoulos, F.; Lenaghan, C.; McEwen, R.; Bradford, J.; Polanski, R.; Ellston, R.; Avivar-Valderas, A.; Pilling, J.; Staniszewska, A.; Cumberbatch, M.; Critchlow, S. E.; Cruzalegui, F.; Barry, S. T., Inhibiting PI3Kbeta with AZD8186 Regulates Key Metabolic Pathways in PTEN-Null Tumors. *Clin Cancer Res* **2017,** 23, (24), 7584-7595.
21. Provenzano, P. P.; Hingorani, S. R., Hyaluronan, fluid pressure, and stromal resistance in pancreas cancer. *Br J Cancer* **2013,** 108, (1), 1-8.
22. Pihlak, R.; Weaver, J. M. J.; Valle, J. W.; McNamara, M. G., Advances in Molecular Profiling and Categorisation of Pancreatic Adenocarcinoma and the Implications for Therapy. *Cancers (Basel)* **2018,** 10, (1).
23. Cairns, R. A.; Harris, I. S.; Mak, T. W., Regulation of cancer cell metabolism. *Nat Rev Cancer* **2011,** 11, (2), 85-95.
24. Dunn, S.; Lombardi, O.; Lukoszek, R.; Cowling, V. H., Oncogenic PIK3CA mutations increase dependency on the mRNA cap methyltransferase, RNMT, in breast cancer cells. *Open Biol* **2019,** 9, (4), 190052.
25. Chalhoub, N.; Baker, S. J., PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol* **2009,** 4, 127-50.
26. Xu, Z.; Han, X.; Ou, D.; Liu, T.; Li, Z.; Jiang, G.; Liu, J.; Zhang, J., Targeting PI3K/AKT/mTOR-mediated autophagy for tumor therapy. *Appl Microbiol Biotechnol* **2020,** 104, (2), 575-587.
27. Heras-Sandoval, D.; Perez-Rojas, J. M.; Hernandez-Damian, J.; Pedraza-Chaverri, J., The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. *Cell Signal* **2014,** 26, (12), 2694-701.
28. Le Grand, J. N.; Chakrama, F. Z.; Seguin-Py, S.; Fraichard, A.; Delage-Mourroux, R.; Jouvenot, M.; Boyer-Guittaut, M., GABARAPL1 (GEC1): original or copycat? *Autophagy* **2011,** 7, (10), 1098-107.
29. Engelman, J. A.; Luo, J.; Cantley, L. C., The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* **2006,** 7, (8), 606-19.
30. Cantley, L. C., The phosphoinositide 3-kinase pathway. *Science* **2002,** 296, (5573), 1655-7.
31. Samuels, Y.; Wang, Z.; Bardelli, A.; Silliman, N.; Ptak, J.; Szabo, S.; Yan, H.; Gazdar, A.; Powell, S. M.; Riggins, G. J.; Willson, J. K.; Markowitz, S.; Kinzler, K. W.; Vogelstein, B.; Velculescu, V. E., High frequency of mutations of the PIK3CA gene in human cancers. *Science* **2004,** 304, (5670), 554.
32. Weng, L. P.; Smith, W. M.; Dahia, P. L.; Ziebold, U.; Gil, E.; Lees, J. A.; Eng, C., PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. *Cancer Res* **1999,** 59, (22), 5808-14.
33. Carracedo, A.; Pandolfi, P. P., The PTEN-PI3K pathway: of feedbacks and cross-talks. *Oncogene* **2008,** 27, (41), 5527-41.
34. Cuesta, C.; Arevalo-Alameda, C.; Castellano, E., The Importance of Being PI3K in the RAS Signaling Network. *Genes (Basel)* **2021,** 12, (7).
35. Castellano, E.; Downward, J., RAS Interaction with PI3K: More Than Just Another Effector Pathway. *Genes Cancer* **2011,** 2, (3), 261-74.
36. Fruman, D. A.; Chiu, H.; Hopkins, B. D.; Bagrodia, S.; Cantley, L. C.; Abraham, R. T., The PI3K Pathway in Human Disease. *Cell* **2017,** 170, (4), 605-635.

37. Pacold, M. E.; Suire, S.; Perisic, O.; Lara-Gonzalez, S.; Davis, C. T.; Walker, E. H.; Hawkins, P. T.; Stephens, L.; Eccleston, J. F.; Williams, R. L., Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. *Cell* **2000,** 103, (6), 931-43.
38. Jones, S. M.; Klinghoffer, R.; Prestwich, G. D.; Toker, A.; Kazlauskas, A., PDGF induces an early and a late wave of PI 3-kinase activity, and only the late wave is required for progression through G1. *Curr Biol* **1999,** 9, (10), 512-21.
39. Cox, A. D.; Der, C. J., The dark side of Ras: regulation of apoptosis. *Oncogene* **2003,** 22, (56), 8999-9006.
40. Hollestelle, A.; Nagel, J. H.; Smid, M.; Lam, S.; Elstrodt, F.; Wasielewski, M.; Ng, S. S.; French, P. J.; Peeters, J. K.; Rozendaal, M. J.; Riaz, M.; Koopman, D. G.; Ten Hagen, T. L.; de Leeuw, B. H.; Zwarthoff, E. C.; Teunisse, A.; van der Spek, P. J.; Klijn, J. G.; Dinjens, W. N.; Ethier, S. P.; Clevers, H.; Jochemsen, A. G.; den Bakker, M. A.; Foekens, J. A.; Martens, J. W.; Schutte, M., Distinct gene mutation profiles among luminal-type and basal-type breast cancer cell lines. *Breast Cancer Res Treat* **2010,** 121, (1), 53-64.
41. Suwa, H.; Yoshimura, T.; Yamaguchi, N.; Kanehira, K.; Manabe, T.; Imamura, M.; Hiai, H.; Fukumoto, M., K-ras and p53 alterations in genomic DNA and transcripts of human pancreatic adenocarcinoma cell lines. *Jpn J Cancer Res* **1994,** 85, (10), 1005-14.
42. Berrozpe, G.; Schaeffer, J.; Peinado, M. A.; Real, F. X.; Perucho, M., Comparative analysis of mutations in the p53 and K-ras genes in pancreatic cancer. *Int J Cancer* **1994,** 58, (2), 185-91.
43. Cayron, C.; Guillermet-Guibert, J., The type of KRAS mutation drives PI3Kalpha/gamma signalling dependency: Implication for the choice of targeted therapy in pancreatic adenocarcinoma patients. *Clin Res Hepatol Gastroenterol* **2021,** 45, (1), 101473.
44. Runnebaum, I. B.; Nagarajan, M.; Bowman, M.; Soto, D.; Sukumar, S., Mutations in p53 as potential molecular markers for human breast cancer. *Proc Natl Acad Sci U S A* **1991,** 88, (23), 10657-61.
45. Wasielewski, M.; Elstrodt, F.; Klijn, J. G.; Berns, E. M.; Schutte, M., Thirteen new p53 gene mutants identified among 41 human breast cancer cell lines. *Breast Cancer Res Treat* **2006,** 99, (1), 97-101.
46. Ruggeri, B.; Zhang, S. Y.; Caamano, J.; DiRado, M.; Flynn, S. D.; Klein-Szanto, A. J., Human pancreatic carcinomas and cell lines reveal frequent and multiple alterations in the p53 and Rb-1 tumor-suppressor genes. *Oncogene* **1992,** 7, (8), 1503-11.
47. Levine, A. J.; Puzio-Kuter, A. M., The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science* **2010,** 330, (6009), 1340-4.
48. Moore, P. S.; Sipos, B.; Orlandini, S.; Sorio, C.; Real, F. X.; Lemoine, N. R.; Gress, T.; Bassi, C.; Kloppel, G.; Kalthoff, H.; Ungefroren, H.; Lohr, M.; Scarpa, A., Genetic profile of 22 pancreatic carcinoma cell lines. Analysis of K-ras, p53, p16 and DPC4/Smad4. *Virchows Arch* **2001,** 439, (6), 798-802.
49. Vora, S. R.; Juric, D.; Kim, N.; Mino-Kenudson, M.; Huynh, T.; Costa, C.; Lockerman, E. L.; Pollack, S. F.; Liu, M.; Li, X.; Lehar, J.; Wiesmann, M.; Wartmann, M.; Chen, Y.; Cao, Z. A.; Pinzon-Ortiz, M.; Kim, S.; Schlegel, R.; Huang, A.; Engelman, J. A., CDK 4/6 inhibitors sensitize PIK3CA mutant breast cancer to PI3K inhibitors. *Cancer Cell* **2014,** 26, (1), 136-49.
50. Cui, L. H.; Li, C. X.; Zhuo, Y. Z.; Yang, L.; Cui, N. Q.; Zhang, S. K., Saikosaponin d ameliorates pancreatic fibrosis by inhibiting autophagy of pancreatic stellate cells via PI3K/Akt/mTOR pathway. *Chem Biol Interact* **2019,** 300, 18-26.
51. Yang, J.; Pi, C.; Wang, G., Inhibition of PI3K/Akt/mTOR pathway by apigenin induces apoptosis and autophagy in hepatocellular carcinoma cells. *Biomed Pharmacother* **2018,** 103, 699-707.
52. Claude-Taupin, A.; Codogno, P.; Dupont, N., Links between autophagy and tissue mechanics. *J Cell Sci* **2021,** 134, (17).
53. King, J. S.; Veltman, D. M.; Insall, R. H., The induction of autophagy by mechanical stress. *Autophagy* **2011,** 7, (12), 1490-9.
54. Totaro, A.; Panciera, T.; Piccolo, S., YAP/TAZ upstream signals and downstream responses. *Nat Cell Biol* **2018,** 20, (8), 888-899.

55. Kalli, M.; Voutouri, C.; Minia, A.; Pliaka, V.; Fotis, C.; Alexopoulos, L. G.; Stylianopoulos, T., Mechanical Compression Regulates Brain Cancer Cell Migration Through MEK1/Erk1 Pathway Activation and GDF15 Expression. *Front Oncol* **2019,** 9, 992.
56. Chen, Y.; Li, Z.; Ju, L. A., Tensile and compressive force regulation on cell mechanosensing. *Biophys Rev* **2019,** 11, (3), 311-318.
57. Rice, A. J.; Cortes, E.; Lachowski, D.; Cheung, B. C. H.; Karim, S. A.; Morton, J. P.; Del Rio Hernandez, A., Matrix stiffness induces epithelial-mesenchymal transition and promotes chemoresistance in pancreatic cancer cells. *Oncogenesis* **2017,** 6, (7), e352.
58. Verret, B.; Cortes, J.; Bachelot, T.; Andre, F.; Arnedos, M., Efficacy of PI3K inhibitors in advanced breast cancer. *Ann Oncol* **2019,** 30, (Suppl_10), x12-x20.
59. Sheridan, C., Pancreatic cancer provides testbed for first mechanotherapeutics. *Nat Biotechnol* **2019,** 37, (8), 829-831.
60. Lampi, M. C.; Reinhart-King, C. A., Targeting extracellular matrix stiffness to attenuate disease: From molecular mechanisms to clinical trials. *Sci Transl Med* **2018,** 10, (422).
61. Mohammadi, H.; Sahai, E., Mechanisms and impact of altered tumour mechanics. *Nat Cell Biol* **2018,** 20, (7), 766-774.
62. Anders, S.; Huber, W., Differential expression analysis for sequence count data. *Genome Biol* **2010,** 11, (10), R106.
63. Livak, K. J.; Schmittgen, T. D., Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001,** 25, (4), 402-8.