The biomechanical context influences the output signaling, independently of *PIK3CA* mutations in breast cancer cells.

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Abstract: Mechanical stresses including tensile and compressive stresses are ubiquitous in nature, and are now well-recognized as being inherent to the development of most cancers. They are integrated by mechanotransduction in cells. Tensile stress is largely associated with YAP/TAZ pathway activation. However, less is known about signaling induced by compressive stress, the latter arising from extracellular matrix remodeling and local tumor growth. In the present study, we used transcriptomic data obtained after unidirectional compression of wild-type and mutant *PIK3CA* breast cancer cells from Kim et al., 2019. We analyzed in an unbiased manner signatures of cell signaling activation including phosphoinositide 3-kinases (PI3Ks) activity pathway in response to compressive stress. Because we found that PI3K activation occurred upon compression, we studied PI3K isoform-specific pathways using known transcriptional targets of PI3Kα inhibitor (BYL719) or of PI3Kβ inhibitor (AZD8186). Our study provides transcriptomic evidences for the role of PI3K pathway in compression-induced mechanotransduction, through the roles of isoform-specific class I PI3Ks and independently of *PIK3CA* alterations. In a compressive environment, the canonical pathways (YAP/TAZ and Piezo) was not increased, while other targetable mechanisms, such as PI3K signal or autophagy, may provide a proliferative advantage and increased cell resistance to chemotherapies.

Keywords: PI3K-AKT, PI3Kα, PI3Kβ, compressive stress, mechanotransduction, autophagy.

1. Introduction

In tissues, all cells are subjected to mechanical forces, exerting a stress on the cell surface, cytoplasm or nucleus. Physically, this stress is measured with internal tissue/cell resistance to deformation described in (N/m^2) or Pascals (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 stress arising from blood flow which physically stresses blood vessel walls. In contrast, tensile and compressive mechanical stresses are inherent of an organ. In physiological condition, cells are subjected to minimal compression stresses. In solid tumor development, compression induced by cell contacts and interactions is increased, as tumor grow in a limited space. The impact of compressive stress on tumor progression and migration are dependent on the magnitude, duration and direction of applied forces, and is associated to extracellular tissue components [1]. In vitro, in most cases, 3D models of solid cancer cells under compressive stress show a cell proliferation decrease. However, compression can increase cancer cell invasive capabilities as well as their resistance to chemotherapeutic treatments due to a continued cell survival and proliferation [3-5]. Currently, there is no mean to predict what will be the cellular output of compression in cancer cells (decreased or increased proliferation and migration).

Once sensed by cells, a mechanical stress induces a mechanotransduction response, coupled to alteration of gene expression, which was largely associated with Hippo pathway activation under tensile stress [6]. In tumors, Hippo pathway containing transcriptional regulators YAP/TAZ can reprogram cancer cells into cancer stem cells and incite tumor initiation, progression and metastasis [6]. 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 [7]. These pathways were very well studied individually. However, in the last decade, research has shown the interconnections of signaling pathways, and key pathways involved in mechanotransduction were noticed [8]. In this context, the importance of phosphoinositide 3-kinases (PI3Ks) activity in mechanotransduction in cancers was misestimated. Recent evidence showed their implication as a pivotal role in mechanotransduction [8].

PI3K proteins can be divided into three classes (I–III) based on their primary structure, regulation, and in vitro lipid substrate specificity [9]. In the literature, the term PI3K refers usually to class I PI3K. This class, composed of four enzymes (α, β, δ, γ), with nonredundant functions [9, 10] and nonredundant roles in cancer [11], generates phosphatidylinositol 3,4,5-trisphosphate (PtdIns-3,4,5-P3 or PIP3) from phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P2 or PIP2) [9]. 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 autostimulation loop [4, 12]. Class I PI3Ks are known to be upstream activators of YAP/TAZ transcriptional pathway under tensile stress, positioning class I PI3Ks proteins as upstream regulators of an essential mechanotransduction signaling [13]. Furthermore, in breast cancer cells, in vivo overexpression of PI3Kβ sensitizes untransformed cells to YAP/TAZ-induced oncogenicity [13]. Furthermore, 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 [14]).

While the role of tensile stress is now largely recognized as affecting tumor cell fate [15], the role of compression stress is emerging. In particular, the global cell signaling modification in response to compression has been poorly characterized. Hence, we decided to use the

transcriptomic data obtained after unidirectional compression of breast cancer cells available to date (Kim et al., 2019 [16] to analyze in an unbiaised manner the 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 [17]) or of PI3Kβ inhibitor (AZD8186) (Lynch et al., 2017 [18]). Here, we confirmed that PI3K pathway merits a greater attention as mechanical sensor of compression and that breast cancer cells are excellent models to study overexpression and activation of PI3K signaling [8].

2. Results

2.1 PI3K-AKT is a key mechanosensitive pathway under compression in breast cancer cells

We first aimed at analyzing how compressive stress altered 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. [16] performed breast cancer cell and cancer associated fibroblast (CAF) compressions 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 [19]. Here, we investigated compression-induced mechanotransduction in cancer cells. Interestingly, in order to elucidate the gene expression evolution of breast cancer cells under compression, Kim et al. based their analysis on two cancer cell lines with two different genetic alteration profiles. The MDA-MB-231 cell line with a PI3K wild-type catalytic domain (*PIK3CA*WT) was compared to MCF-7 containing a constitutive PI3K activation (*PIK3CA*E545K). Both breast cancer cell lines were compressed 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. 1A). Gene signatures in different pathways were normalized into transcripts per million and compared to housekeeping gene expression such as ACTINB, LAMINA1 and LAMIN gene class. The expressions of the latter were not affected by increasing compression (Figure. S1A). In wild-type PI3KCA MDA-MB-231 cells submitted to high compression (8kPa), the NOTCH, MAPK and PI3K-AKT were found as most enriched pathways compared to no compressed condition (0 kPa) (Figure. 1B), showing that few genes belonging to these signature had significant increased expression. This pathway enrichment was not found in MCF-7 cells presenting a constitutively activated PI3Kα. In addition, under increasing compression [0], [0.3-0.8], [1.5-4.0], [7.0-8.0] kPa; NOTCH, MAPK, and PI3K-AKT signatures mean gene expression (from GSEA canonical signatures) were significantly overexpressed only for MAPK and PI3K-AKT pathways in wild-type *PIK3CA* MDA-MB-231 cells compared to constitutively active PI3K MCF-7 cells (Figure. 1C), suggesting a global overexpression of all genes belonging to these pathways. Contrariwise, MCF-7 cells showed a significant decrease for NOTCH and MAPK pathway gene expressions under increasing compressive stress (Figure. 1C). However and surprisingly, YAP/TAZ pathway was not significantly affected in MCF-7 and MDA-MB-231 cells (Figure. S1B). Taken together, these data showed a sensitivity (increasing gene expression) to growing compression (from 0 to 8 kPa) in wild-type *PI3KCA* cells compared to overactivated *PIK3CA* cells.

This sensitivity in signaling pathway expression adds an additional link to demonstrate general PI3K involvement in mechanotransduction.

2.2 PI3Kα/β are sensitive to compression in breast cancer cells

To investigate the specific role of PI3K isoforms, we analyzed data from two studies which used selective inhibitors of PI3K isoforms: Bosch et al., 2015 [17] and Lynch et al., 2017 [18]. The authors used BYL719 and AZD8186 compounds to selectively inhibit PI3Kα or PI3Kβ in mutant *PIK3CA* MCF-7 and mutant PTEN HCC70 breast cancer cells respectively (Figure. 1A). We know that mutant PTEN leads to increased PI3K activity. Gene expression data from PI3Kα inhibition in MCF-7 and PI3Kβ inhibition in HCC70 cells were used to define PI3Kα and PI3Kβ signatures. Selective transcriptional targets were crossed-compared with compression-specific transcriptional targets identified from Kim et al. [16] (Figure. 1A).

We analyzed data after PI3Kα inhibition and we observed 1279 genes with a significantly altered expression in MCF-7. 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 GSEA 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 to 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. We next found that both PI3Kα and PI3Kβ gene signatures (after PI3Kα or PI3Kβ inhibitions) were significantly overexpressed under increasing compression in wild-type PI3K MDA-MB-231 cells, but significantly decreased in PI3K oncogenically activated MCF-7 cells (Figure. 3).

These analyses showed 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*.

2.3 Compression influences autophagy gene expression in breast cancer cells

PI3Kα and PI3Kβ are known as regulators of autophagy process [20, 21]. When comparing PI3Kα and PI3Kβ signatures, PI3K-AKT GSEA canonical pathway and compressive stress selective gene expression, only GABA type A receptor associated protein like 1 (GABARAPL1) gene was significantly affected and overlap in these four signatures (Figure. 2). Briefly, this gene encodes for a structural protein of autophagosome and autophagolysosome involved in autophagy process (reviewed in [22]). 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. 3B), 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. 3C).

Therefore, autophagy GSEA pathway and GABARAPL1 gene expressions were well correlated and overexpressed in wild-type *PIK3CA* MDA-MB-231 cells upon compression.

Figures, Tables and Schemes

Figure 1. PI3K-AKT: a pathway involved in mechanotransduction in breast cancer cells. A. On the left side, the canonical method consisting in Gene Set Enrichment Analysis (GSEA) using canonical signatures. Available transcriptomic data from compressed breast cancer cells were used (Gene Expression Omnibus data (GEO) Kim et al., 2019. 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. In parallel, differential expression analysis were 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 compressive stress study. B. MDA-MB-231 (*PIK3CA*wt) cells were compressed using [7.0 to 8.0 kPa] high compressive stress(High) compared to no compression (None). GSEA KEGG NOTCH (47 genes), MAPK (264 genes) signaling pathways and REACTOME PI3K-AKT (102 genes) signaling in cancer were analyzed. p-values<0.05. NES: Normalized Enrichment Score; FDR: Fold Discovery Rate. C. 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]) and gene expression was quantified using Agilent microarray (Data available in https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133134). Differential expression analysis was performed and this data were correlated to GSEA KEGG NOTCH (47 genes), MAPK (264 genes) signaling pathways and REACTOME PI3K-AKT signaling in cancer (102 genes). ± SEM. *<0.05; **<0.01; ***<0.001.

Figure 2. Comparative analysis between compressive stress, PI3Kα/PI3Kβ signatures and REACTOME PI3K-AKT signaling in cancer. 1279, 933 and 1052 differentially expressed genes in PI3Kα signature, PI3Kβ signature, compressive stress and 102 genes of REACTOME PI3K-AKT signaling in cancer respectively, were compared to investigate overlapping. Venn diagram show 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 by PI3Kα inhibition/PI3Kβinhibition/compressive stress was GABARAPL1 (highlighted in yellow).

Figure 3. Increased compressive stress influences PI3K and autophagy signaling pathways in breast cancer cells. MDA-MB-231 (*PIK3CA*wt; blue triangles) 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). A. Differentially expressed genes (p-value<0.05) were correlated to PI3Kα/β signatures (PI3Kα signature (1279 genes): after BYL719 inhibition; PI3K β signature (933 genes): after AZD8186 inhibition). B. GABRAPL1 (1 gene) and autophagy (35 genes). ± SEM. *<0.05; ***<0.001.

3. Discussion

Results obtained in the current work reinforce the PI3K impact in mechanotransduction in cancer cells, the possible differential role of PI3K isoform specific signaling and the necessity to test the effect of PI3Kα/β inhibitors in cancers submitted to compressive stress independently of their mutational status.

The PI3K pathway is essential for cell proliferation, survival, and metabolism [23, 24]. In human cancers, the PI3KCA gene is frequently mutated in particular in hot spot mutations that reside in the p110α helical domain (E542K and E545K) found in over a third of estrogen receptor (ER)-positive breast cancer, representing the most common genomic alteration in this group of tumors [25]. A large number of studies describe the effect of PI3Kα/β inhibitions in different cancer models [10]. However, few high throughput sequencing data are available after PI3K inhibition and compressive stress. This limitation constrained our study to the analysis of different breast cancer cell lines in order to compare the combined effects of the PI3Kα/β inhibitions and compressive stress. In our comparative study, two breast cancer cell lines were compared: MCF-7 containing a constitutive PI3Kα activation (*PIK3CA*E545K), and MDA-MB-231 with a wild-type *PIK3CA* and containing a high level of PTEN and low level of phosphorylated AKT [26]. PTEN phosphatase possesses a PI3K antagonist action balancing the activation of the canonical AKT-mTORC1 pathway [27]. Therefore, comparing MCF-7 and MDA-MB-231 allows to compare two cell lines with high and low activation of PI3K-AKT-mTORC1 axis respectively. We found that compressive stress affects PI3K-AKT pathway gene expression in *PI3KCA*wt cells, in contrast with constitutively active PI3Kα cell line. Recent evidence demonstrates that PI3K enzymes are essential in mechanotransduction and we previously suggested that PI3K functions as a hub in mechanotransduction in pancreatic and breast cancers [8]. These data reinforce the importance of studying the coupling between mechanical stress and genetic alterations skewing pro-oncogenic cell signaling.

Increasing unidirectional compressive stress induced an overexpression of PI3Kα and PI3Kβ gene signatures. The PI3K isoform activation is notably cancer type- and context-dependent. In breast cancer cells, the increasing compression influence both the PI3Kα/β pathways gene expression, that are known to have different signaling outputs [28]. Our comparative analysis revealed that 1279 genes were significantly regulated after PI3Kα inhibition, 933 after PI3Kβ inhibition and 1052 in compressive stress condition, therefore 139 commonly regulated when inhibiting PI3Kα and PI3Kβ. These data reinforce the importance of studying the isoform specificity of PI3K in a compressive stress environment.

Finally, by comparing PI3Kα and 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. GABARAPL1 encodes for a protein 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 [22]). Autophagy is a highly conserved

catabolic process, participating in the balanced 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 [29, 30]. This pathway is known as a key pathway in therapeutic strategy for a variety of tumors. Further, we know that induction of autophagy is correlated with mechanical stress [20, 31]. In Dictyostelium discoideum, an organism which do not present any homolog of vertebrate Class II PI3K, compression activates autophagy in a mTORC1 independent manner [32]. This mechanism, if confirmed in mammalian cells, could participate in cancer cell survival as well as resistance to chemical treatments.

Under increasing compression, gene expression signatures of different protumoral signaling pathways were significantly enriched. However and surprisingly, YAP/TAZ via Hippo or No Hippo pathway, known as key regulator of mechanotransduction under tensile stress [33], was not significantly affected in both *PIK3CA*E545K and *PIK3CA*WT breast cancer cells under unidirectional compressive stress (Figure. S1B). In compressive stress context, the implication of YAP/TAZ pathway was less investigated. It was also 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 [34, 35]. In Kim et al. 2019, cells were compressed in vitro using alginate disk and compression cylinder. The tension context, due to cell-cell or cell-coating adhesions, is almost absent in these experimental conditions [16]. However, a stress associated with cytoskeleton remodeling cannot be completely ruled out in these conditions. The exact type of mechanical stress and the cell-dependent induction of cytoskeleton is key to understand cell mechanotransduction processes, as cells adapt to them environmental stresses. Further, class I PI3Ks were described as upstream activators of the YAP/TAZ pathway; tensile stress is permissive for their control of YAP/TAZ and targeting of PI3K could be a novel strategy to hinder the potential YAP/TAZ oncogenic dependence [8]. This PI3K activation was described in tensile stress conditions, however our transcriptional analysis does not seem to confirm those finding in the context of unidirectional compressive stress.

In breast cancer, compressive stress is likely to occur as it was associated with large fibrosis and extracellular matrix (ECM) deposits [36]. Similarly, in pancreatic cancer, there is an important influence of tumoral microenvironment possessing a variable stiffness and variable interstitial pressure depending on the area in pancreas and progress statement of disease [37]. In addition to compressive stress induced by ECM, ECM also influences the desmoplastic reaction via epithelial-mesenchymal transition (EMT) and further resistance to chemotherapeutic agents [38]. Because those high compressive stress occurs in breast cancer [39] but also in pancreatic ductal adenocarcinomas [12], our study should be extended to other types of solid cancers.

4. Conclusion

In addition to the canonical pathways (YAP/TAZ and Piezo) associated with mechanical stresses, our study provides transcriptomic evidences for the role of PI3K-AKT pathway in compression-induced mechanotransduction, through class I PI3Ks (PI3Kα-PI3Kβ) roles. Further, other mechanisms inherent to compressive stress, such as autophagy induction, may provide a proliferative advantage and increase cell resistance in compression stress environment. Finally, oncogenic alterations are known to skew normal cell signaling. However, 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

Differential expression analysis after compressive stress in MCF-7 and MDA-MB-231 were performed from data provided by Kim et al., 2019 [16]. 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) [17] 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/) [18]. For compressive stress and PI3Kα and PI3Kβ inhibition signatures, differential expression analysis were performed using DESeq2 v1.26.0 [40] 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). *<0.05; **<0.01; ***<0.001. Enrichement analysis were performed using Gene Set Enrichement Analysis software (GSEA), p-value <0.05 and Fold Discovery Rate (FDR)<25%.

Supplementary Materials:

Figure S1. Housekeeping genes and YAP/TAZ pathway under increasing compressive stress in breast cancer cells. MDA-MB-231 (*PIK3CA*wt; blue triangles) 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. Mechanoregulation and pathology of YAP/TAZ via HIPPO and non HIPPO mechanisms (45 genes). ± SEM. *<0.05; **<0.01; ***<0.001.

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Data Availability Statement:

For this work, we utilized transcriptomic data from compressed breast cancer cells from Kim et al., 2019 [16], from Gene Expression Omnibus GEO accession number: GSE133134. Our work

cor-relates compression data to PI3Kα inhibition data from Bosch et al., 2015 [17], GEO accession number: GSE64033) or PI3Kβ inhibition data from Lynch et al., 2017 [18], ArrayExpress: ENA - ERP015852).

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Conflicts of Interest: The authors disclose no conflicts.

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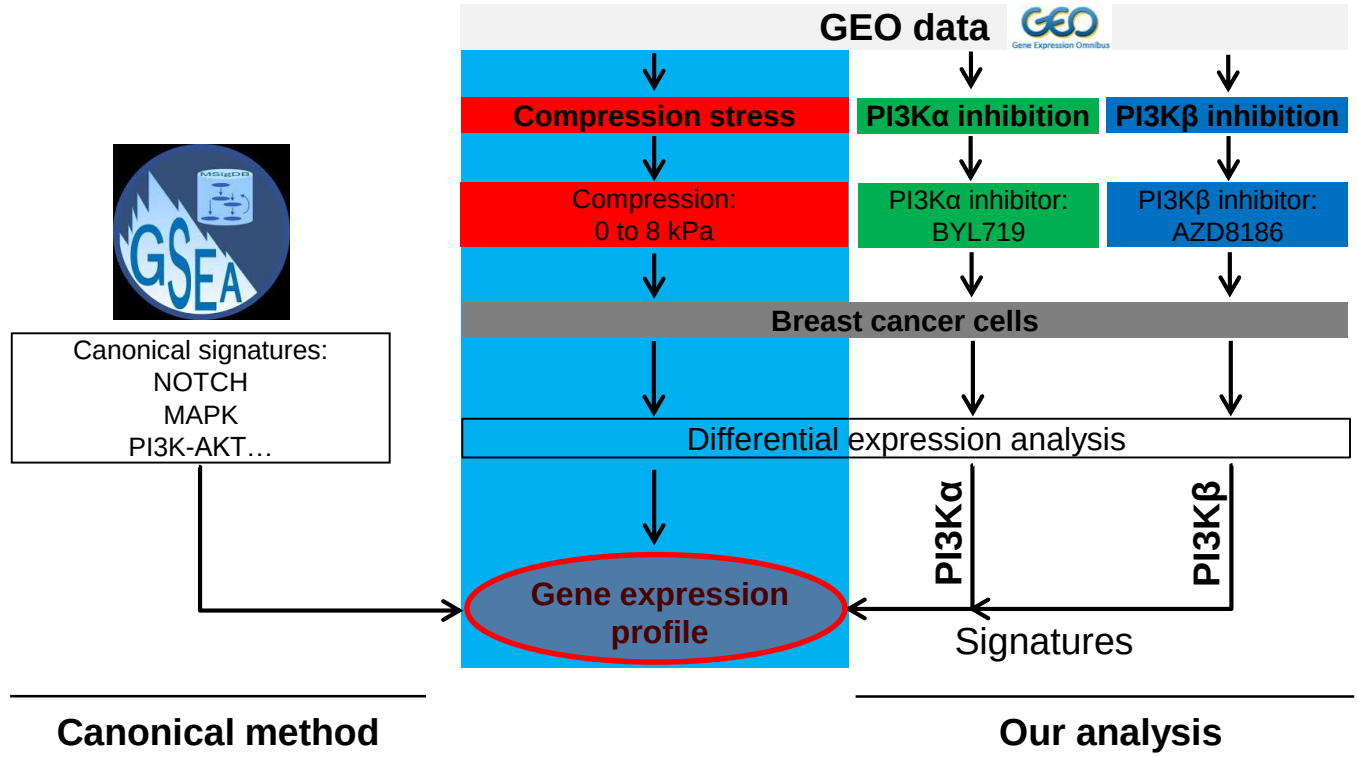

A
GEO data
Compression stress
PI3Kα inhibition
PI3Kβ inhibition
Compression: 0 to 8 kPa
PI3Kα inhibitor: BYL719
PI3Kβ inhibitor: AZD8186
Breast cancer cells
Differential expression analysis
Canonical signatures:
NOTCH
MAPK
PI3K-AKT…
Gene expression profile
PI3Kα
PI3Kβ
Signatures
Canonical method
Our analysis

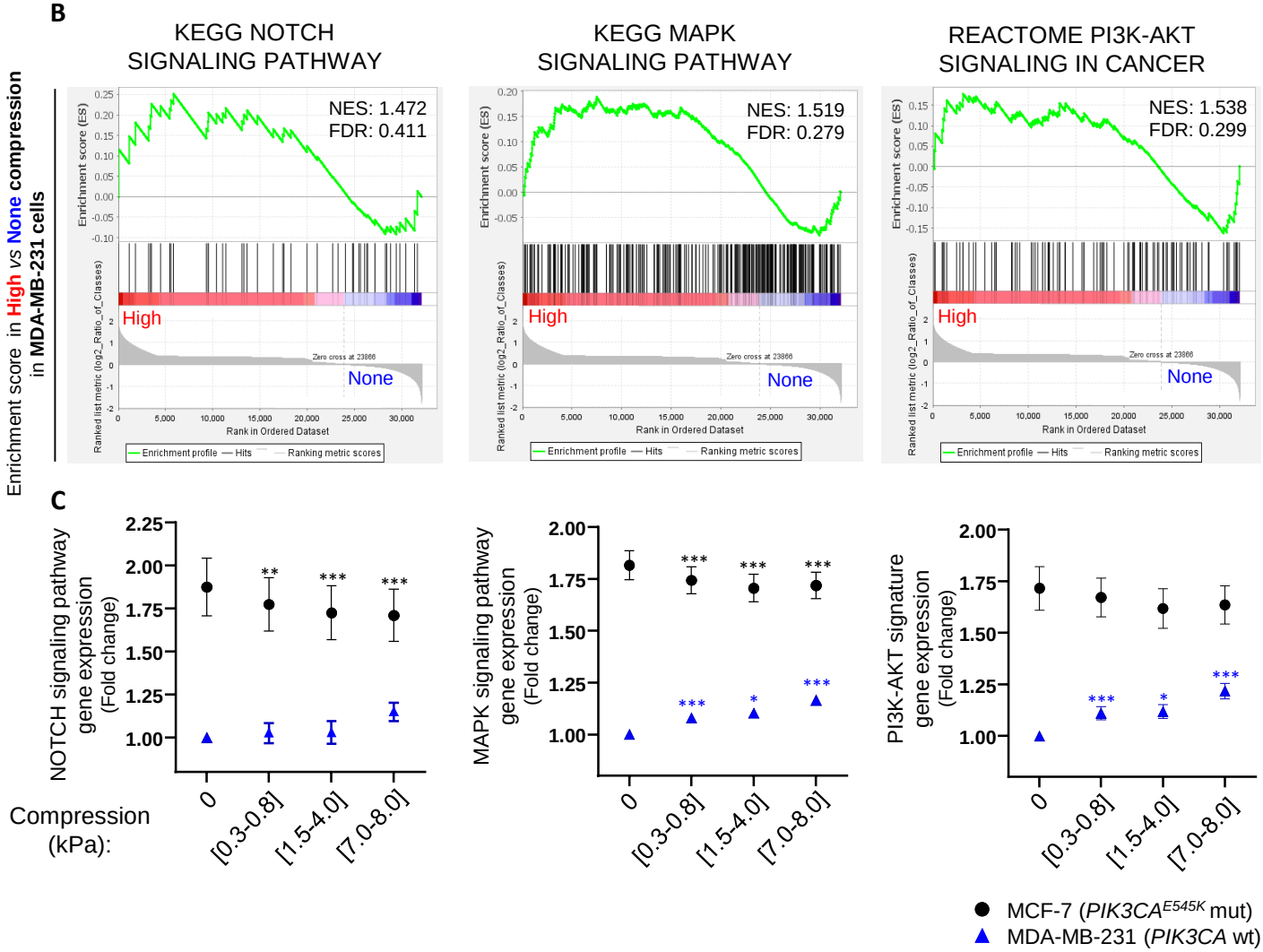
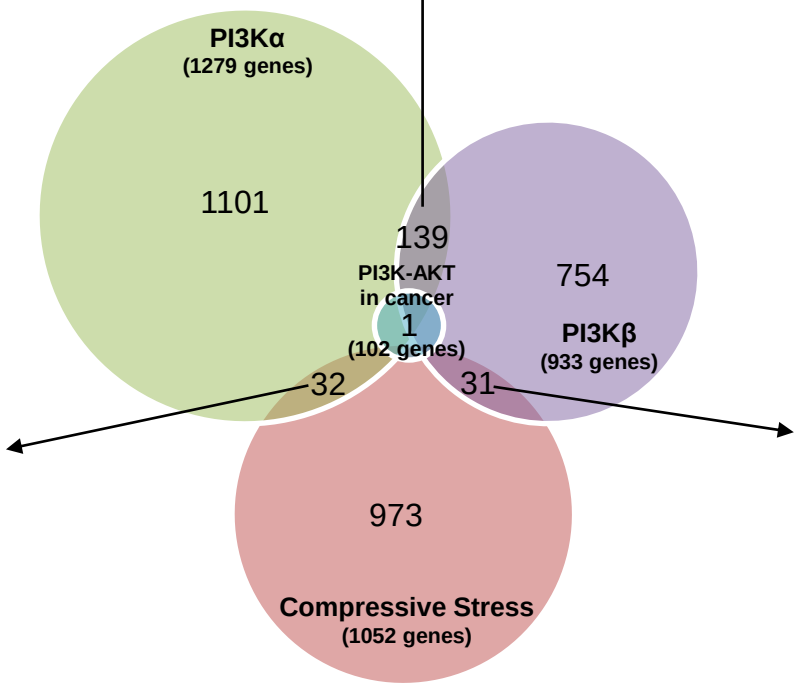

B
KEGG NOTCH SIGNALING PATHWAY
KEGG MAPK SIGNALING PATHWAY
REACTOME PI3K-AKT SIGNALING IN CANCER
Enrichment score in High vs None compression in MDA-MB-231 cells
NES: 1.472
FDR: 0.411
NES: 1.519
FDR: 0.279
NES: 1.538
FDR: 0.299
Enrichment score (ES)
Ranked list metric (log2_Ratio_of_Classes)
High
None
Zero cross at 23866
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores
C
NOTCH signaling pathway gene expression (Fold change)
MAPK signaling pathway gene expression (Fold change)
PI3K-AKT signature gene expression (Fold change)
Compression (kPa):
0
[0.3-0.8]
[1.5-4.0]
[7.0-8.0]
MCF-7 (PIK3CA E545K mut)
MDA-MB-231 (PIK3CA wt)

Figure 2

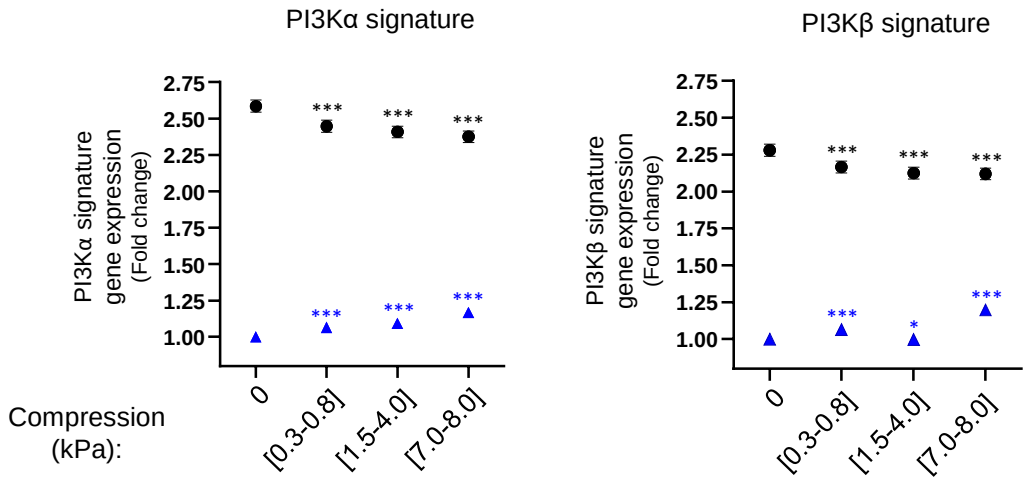
PI3Kα/PI3Kβ							
GABARAPL1	C16orf61	CENPB	EIF3B	GRWD1	MRPL14	NUP54	PSMD12
ABCA1	C16orf80	CIRH1A	ELF3	GTPBP4	MRPL3	NXT1	PSMG1
ABCE1	C16orf87	COL12A1	FABP5	HBP1	MRPL36	ODC1	PSMG3
ABCF2	C1orf135	CORO1C	FAM134B	HIGD1A	MRTO4	OTUD1	PTS
ACAT2	C20orf24	CRBN	FAM49B	HMGCS1	MVD	PAK1IP1	RANBP1
ACLY	C3orf14	CSE1L	FBXO32	HSD17B12	MYLIP	PCMTD1	RAP2C
ADSL	C5orf41	CTDSP2	FDFT1	HSPA4	NDUFC1	PCMTD2	RB1CC1
AKR1C3	C7orf41	CTPS	FDPS	IDI1	NIP7	PDK4	RBL2
ALDH1B1	CA2	DDIT4	FH	IMP4	NIPA2	PDSS1	RDH11
AMD1	CALCOCO1	DEGS1	FOXO1	INSIG1	NME1	PHF23	RHOC
ATAD3A	CCNE1	DHCR24	FOXO4	IRS2	NOP2	PHLDA2	RNASEH1
BCAS1	CCNG2	DHCR7	GABPB1	JMY	NOP56	PIK3IP1	RPF2
BOLA3	CCRN4L	DKC1	GBP2	KIAA0430	NOP58	PIM1	RPL39L
BRD8	CCT2	DSCC1	GCH1	KLHL24	NPHP3	PNO1	
BRI3BP	CCT7	DUSP14	GGCT	LDLR	NSDHL	PPAT	
BRIX1	CDC42EP2	DYNLL1	GLRX2	LRP8	NSUN2	PPIL1	
BYSL	CDK5RAP3	EBP	GRB2	MAGEF1	NUMA1	PPP2CA	
C13orf15	CENPB	EIF2B3	GRB7	MCM10	NUP35	PSMC2	



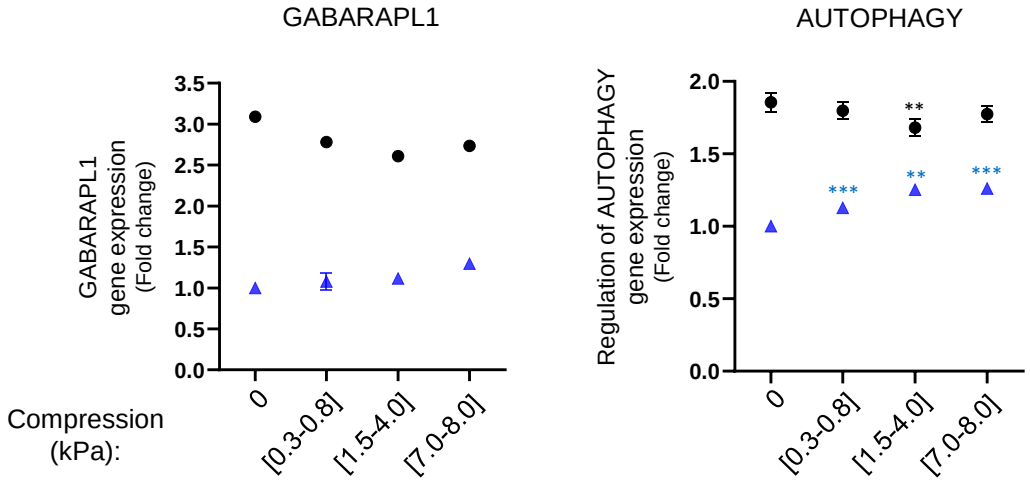
Comp stress/PI3Kα
GABARAPL1
AK2
CACYBP
CDC25A
CDH26
CHORDC1
CXCR7
CYP26B1
DOLK
EIF5
FLJ10661
GATS
KREMEN2
LCMT2
LFNG
MRPL1
PARD6G
PDE12
POLR3H
RBM14
RIN2
RNU1-5
RPUSD2
RTN4IP1
SCARNA13
SEMA6C
SESTD1
SNORA12
SPATA7
SPRED1
SYTL4
TTC21A

Comp stress/PI3Kβ
GABARAPL1
ANKRD36
C7orf74
CLK4
CTGF
DDX58
ERRFI1
F3
FAM13A
FAS
GDA
INPP4B
LOC100272228
LOC100289230
LSS
MAT2A
P2RX7
PNPLA3
POP1
S100PBP
SESTD1
SNORD86
SPATA7
SYTL4
TIA1
TRAF5
TRANK1
ZNF248
ZNF302
ZNF37BP
ZNF608

A



B



● MCF-7 (*PIK3CA*^{E545K} mut)
▲ MDA-MB-231 (*PIK3CA* wt)