

Spotlight

Cytoskeleton
Architecture Regulates
Glycolysis Coupling
Cellular Metabolism to
Mechanical CuesAlisdair R. Fernie,^{1,2,*}
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Energy-demanding processes, such as cell growth, migration, and differentiation, are tension modulated, begging the question whether metabolism and mechanical tension are tightly linked. A recent report by Park *et al.* shows that stiffness in the extracellular matrix (ECM) promotes reorganization of actin, resulting in enhanced glycolysis.

The physical interaction of glycolysis with cytoskeletal components has long been known. Indeed, components of the cytoskeleton are among the first reported structural elements that interact with glycolytic enzyme–enzyme assemblies, such as those containing phosphofructokinase (PFK), which is widely regarded as one of the key regulatory enzymes of glycolysis [1] and is found in all kingdoms of life [2]. It is often postulated that one of the main reasons underlying PFK-containing assemblies is that they guarantee high rates of ATP production at cellular localities where demand is high. However, the recent study by Park *et al.* [3] reveals another mechanism, where such assembly proves to be a useful point of metabolic control in mediating the response to stiffness in the ECM, a feature occurring during aging or at the onset of disease; this mechanism promotes the reorganization of actin filaments and results in elevated rates of

glycolysis. Alterations in this pathway were also demonstrated to result in enhanced glucose metabolism in human lung tumors.

Cells can sense changes in tension in the ECM that surrounds them by means of integrin and cadherin transmembrane receptors, which provide signals between the cell and its surroundings. These receptors can alter the organization of the actomyosin cytoskeletal network, which is coupled to intracellular signaling pathways, including those that promote cell growth and survival [4]. Contemporaneously to these structural changes, metabolism is boosted to provide the energy and biomass gain required for cell proliferation [5]. Normal cells carefully coordinate the use of glycolysis and oxidative phosphorylation to meet these demands; however, cancer is often characterized by the predominance of glycolysis above mitochondrial respiration [6]. Although accumulating data have started to suggest a link between tension in the ECM and such a metabolic shift, understanding of the mechanistic basis of this link has been lacking.

For their study, Park and coworkers chose human bronchial epithelial cells, which experience mechanical stimuli with every breath and are exposed to particularly high tension in the ECM during pulmonary fibrosis or lung cancer. To experimentally modulate the extracellular environment, the authors plated these cells on either stiff or soft collagen substrates. Dependent on the substrate, the cells displayed distinct morphologies in terms of actomyosin contractility, with cultures on the soft substrates resulting in a downregulation of glycolysis and the tricarboxylic acid (TCA) cycle; this was evidenced by changes in steady-state levels of metabolites and their labeling patterns following feeding with ¹³C glucose. Although glycolysis is often controlled at the level of expression, the authors observed that most enzymes remained unchanged.

However, the expression of all three PFK isoforms were downregulated, as was its extractable enzyme activity. Furthermore, expression of a fluorescently tagged platelet PFK under a strong promoter revealed that the protein level differed between the two culture conditions, indicative of changes conferred by a post-translational mechanism. Given that cancer cells exhibit both variable mechanical conditions, Park *et al.* next tested whether this glycolytic response had a role in cancer progression. Intriguingly, this tension-specific downregulation of glycolysis was overridden in oncogenically transformed cells targeting *TP53*, *KRAS*, and *MYC*, as well as in a panel of patient-derived lung cancer lines [3].

To gain more extensive mechanistic insight and based on the known requirement for actomyosin contractility for mechanosensing [7], Park *et al.* next inhibited myosin II with blebbistatin. This resulted in decreased expression of platelet PFK and also in the glycolytic rate. Moreover, cells plated on low adhesion plates displayed decreased platelet PFK expression and a lower FAK phosphorylation; in addition, cells became rounder in shape and contained shorter fragmented filaments, and there was a shift from fragmented to bundled actin filaments. Application of the actin monomer-sequestering drug, latrunculin A, also decreased both the length of F-actin bundles and the expression of platelet PFK. However, the sensitivity of glycolysis to mechanical stress was diminished in oncotransformed cells, enabling the cancer cells to stabilize energy production in the face of extracellular cues.

Given that the platelet PFK appeared to be post-translationally modified, the authors subsequently tested whether this was the mechanism by which the rate of glycolysis was controlled in response to extracellular tension. To test this, they labeled platelet PFK with L-azidohomoalanine in human embryonic kidney cells and followed

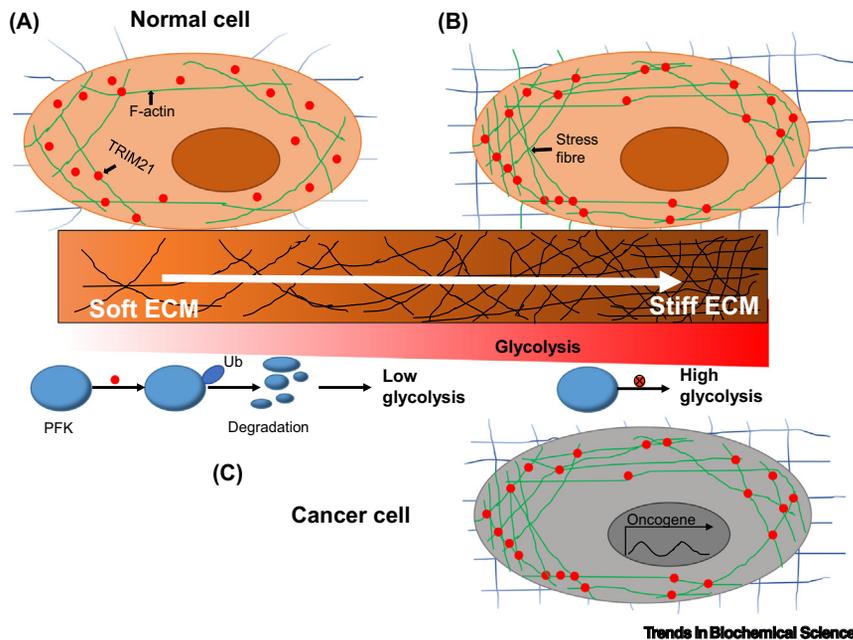


Figure 1. Cancer Cells Hijack a Tension-Induced Glycolytic Machinery. (A) Normal cells (orange) surrounded by soft extracellular matrix (ECM; black lines) contain less-bundled actin filaments (green lines), which facilitates optimal glycolysis by mediating Tripartite motif containing-21 (TRIM21; red sphere)-dependent ubiquitination and subsequent degradation of phosphofructokinase (PFK; blue oval), a glycolytic rate-limiting enzyme. (B) Increased tension in cells due to stiff ECM promotes the formation of highly bundled actin stress fibers, which entrap TRIM21, rendering them inactive and increasing the rate of glycolysis. (C) By contrast, cancer cells (gray) are capable of promoting the formation of actin stress fibers independently of the stiffness of the ECM to elevate the levels of glycolysis. Tensional forces are inherent features of the ECM equivalents in the cell walls of plants and microbes, modulations to which are known to impact cytoskeleton behavior. This could regulate similar metabolic processes to ensure proper growth and response to environmental changes. Abbreviation: Ub, ubiquitin.

protein degradation for 24 h, revealing that, in the presence of the proteasome inhibitor MG132, platelet PFK abundance was increased. Furthermore, by identifying ubiquitinated proteins in cellular extracts, the authors observed that PFK ubiquitylation was enhanced. Intriguingly, mutagenesis of the candidate lysine ubiquitylation sites K281R, K272R, and K275R in platelet, liver, and muscle forms of PFK rescued PFK expression, confirming a conserved regulation of the isoforms via mechanical cues. Given that previous studies have recognized the E3 ligase TRIM21 as the signal targeting proteasomal degradation of platelet PFK [8], this was hypothesized to be a likely regulatory candidate. The authors postulated that

platelet PFK abundance and glycolysis were regulated by thick F-actin bundles, which are able to sequester TRIM21. They put this hypothesis to the test by evaluating the direct alteration of F-actin architecture in the phosphomimetic α -actin mutant ACTN1(Y246E), which displays a strong bundling phenotype [9]. When plated on soft surfaces, these mutant cells were able to sequester fluorescently labeled TRIM21, as well as to maintain enhanced actin bundles, platelet PFK abundance, and glycolysis, confirming that their postulated mechanism is correct.

In summary, Park *et al.* mechanistically linked changes in glycolysis to the organization of the actomyosin cytoskeletal

network in response to mechanical forces. These findings have wider implications across kingdoms because mechanical forces are inherent features of growing cells and their modulation results in similar cytoskeletal responses. Furthermore, the transduction of mechanical forces in other biological systems also relies on the ECM, as seen in the cell walls in plants and bacteria (Figure 1) [10]. Clearly, the work by Park *et al.* opens doors for more exciting interdisciplinary approaches to investigate the link between mechanical and chemical signals.

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