Breaking Cancer's Energy Code: New Insights into Hydrogen Sulfide and Pyruvate Kinase M2 Regulation

Hydrogen sulfide modifies the pyruvate kinase M2 enzyme at cysteine 326, affecting cancer cell metabolism. Blocking this modification reduces PKM2 activity and impairs cancer cell division, leading to complete tumor suppression.

Cancer cells undergo significant metabolic reprogramming that is primarily characterized by the Warburg effect. In this process, they prefer aerobic glycolysis over oxidative phosphorylation. Although this process is less efficient for ATP production, it provides essential metabolic intermediates for rapid cancer cell proliferation under nutrient-limited conditions.

The glycolytic pathway is regulated by three key enzymes, with the pyruvate kinase M2 (PKM2) playing a crucial role. PKM2, which is predominantly expressed in cancer cells, differs from PKM1's catalytic properties. While PKM1 maintains high activity as a tetramer, PKM2's activity is typically low in cancer cells because of various post-translational modifications, which allow redirection of glucose metabolism toward biomass synthesis.

Recent research has focused on hydrogen sulfide (H_2S), an endogenous gasotransmitter that shows concentration-dependent effects on cancer progression. H_2S primarily functions through protein sulfhydration, a post-translational modification where H_2S forms a persulfide (-SSH) bond on cysteine residues of target proteins.[1] At lower concentrations, H_2S promotes tumor growth through multiple mechanisms, including antiapoptotic effects, DNA repair, and angiogenesis. However, higher concentrations can inhibit cancer cell proliferation.

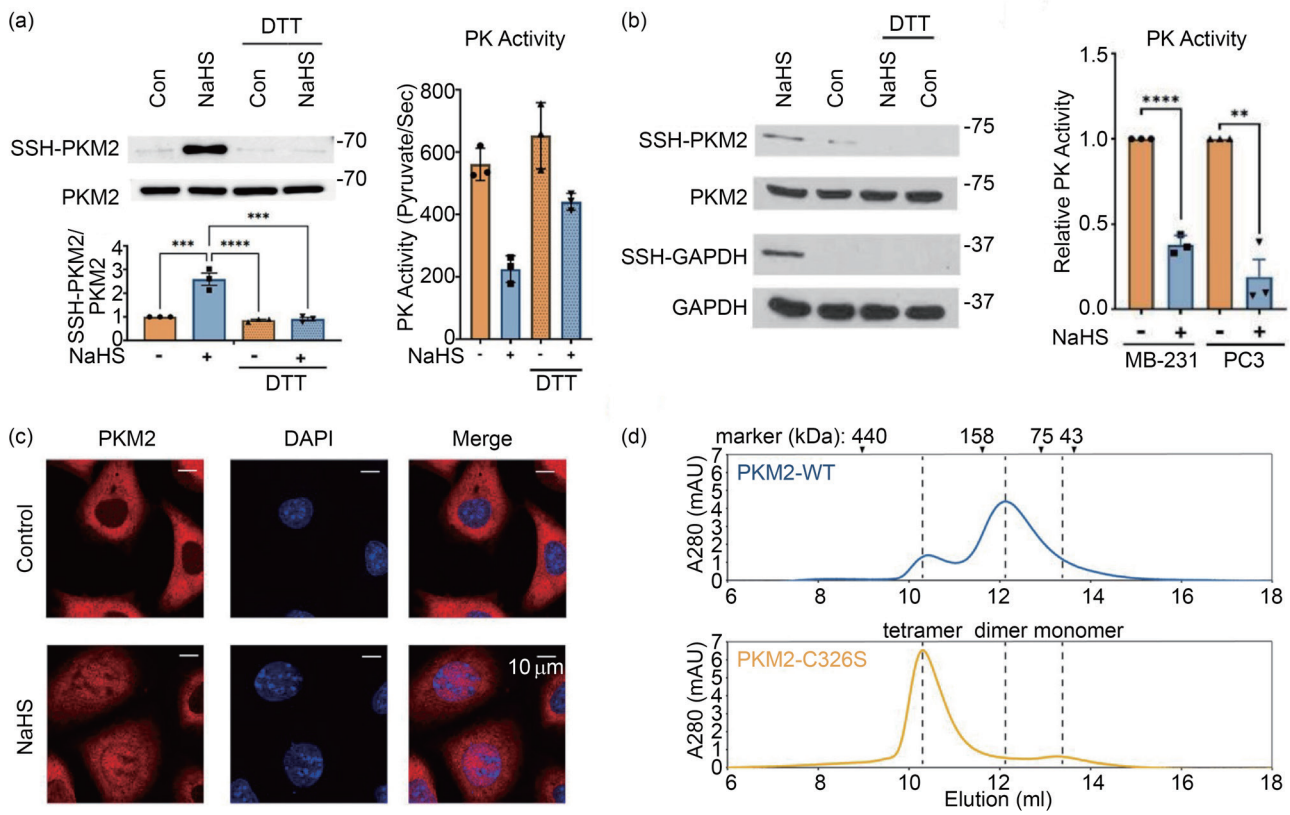

Fig. 1: (a) Results of two main analyses performed in the experimental study. The first part examined PKM2 sulfhydration in MDA-MB-231 cell lysates treated with NaHS and DTT by using a biotin switch assay followed by immunoblotting with an anti-PKM2 antibody. The second part investigated pyruvate kinase activity by measuring pyruvate production in recombinant PKM2 after treatment with NaHS and DTT under ice-cold conditions. (b) Results of the experiment investigating protein sulfhydration and enzymatic activity in two parts. The first part examined PC3 cell lysates treated with NaHS and DTT by using biotin switch assay and immunoblotting to detect sulfhydration of both PKM2 and GAPDH (used as a positive control). The second part focused on measuring pyruvate kinase activity in both MDA-MB-231 and PC3 cell lysates after NaHS treatment, specifically by quantifying pyruvate production. (c) Treatment of MDA-MB-231 cells with NaHS and visualization of PKM2 localization using immunocytochemistry with DAPI nuclear staining (scale: 10 μm). (d) Results of gel filtration analysis of PKM2 proteins (WT and C326S mutant) performed without FBP to examine their oligomeric states. [Reproduced from Ref. 2]

The interplay between H_2S and PKM2 represents a fascinating area of research, particularly given that PKM2 activity can be inhibited by L-cysteine, which serves as the primary source for H_2S production. This intricate connection, which is mediated through protein sulfhydration, reveals a critical regulatory mechanism in cancer cell metabolism. The emerging understanding of how H_2S-mediated protein sulfhydration influences cancer progression has opened promising new avenues for therapeutic interventions that specifically target cancer-specific metabolic pathways. To further explore this novel regulatory mechanism, Lu-Hai Wang (China Medical University), Hui-Chun Cheng (National Tsing Hua University), and Kai-Ti Lin (National Tsing Hua University) launched a collaborative effort to investigate the molecular mechanisms underlying H_2S-mediated PKM2 regulation in cancer cells.

The researchers first investigated the role of H_2S in PKM2 activity through protein sulfhydration. **Figures 1(a) and 1(b)** show that treatment with NaHS, a H_2S donor, induced PKM2 sulfhydration in both breast cancer MDA-MB-231 and prostate cancer PC3 cells; this modification reduced PKM2 enzyme activity, which could be reversed by dithiothreitol (DTT) treatment. Importantly, H_2S caused the dissociation of fructose 1,6-bisphosphate (FBP)-induced PKM2 tetramers into monomers or dimers. This led to enhanced PKM2 nuclear translocation (**Fig. 1(c)**) and increased expression of PKM2-responsive genes, such as cyclin D1 and glutaminase-1; the expression levels of these genes show a positive correlation with tumor growth. When H_2S was depleted using the aminooxyacetic acid inhibitor or by knocking down H_2S-producing enzymes, such as cystathionine β-synthase and cystathionine γ-lyase, both PKM2 sulfhydration and nuclear translocation decreased.

Through mass spectrometry analysis, the researchers identified two sulfhydration sites on recombinant PKM2: cysteines 49 and 326. However, only cysteine 326 was found to be endogenously sulfhydrated in MDA-MB-231 cells. To further study the effects of sulfhydration at this site, they created a mutation replacing cysteine 326 with serine ($PKM2^{C326S}$). This mutation significantly reduced PKM2 sulfhydration and, notably, resulted in increased tetramer formation compared to wild-type PKM2 (**Fig. 1(d)**). Crystal structure analysis revealed that $PKM2^{C326S}$ adopts a unique tetrameric conformation that is different from previously known conformations (**Fig. 2(a)**). The X-ray diffraction data were collected at **TPS 07A** of the NSRRC.

The functional consequences of blocking PKM2 sulfhydration at C326 were substantial. The $PKM2^{C326S}$ mutation increased pyruvate kinase activity and led to significant metabolic changes in cells. Cells expressing $PKM2^{C326S}$ showed increased oxygen consumption rates and enhanced mitochondrial oxidative phosphorylation, while extracellular acidification rates only slightly increased. These cells also showed reduced expression of most PKM2-responsive genes, decreased levels of glycolytic intermediates, and reduced nuclear translocation of PKM2.

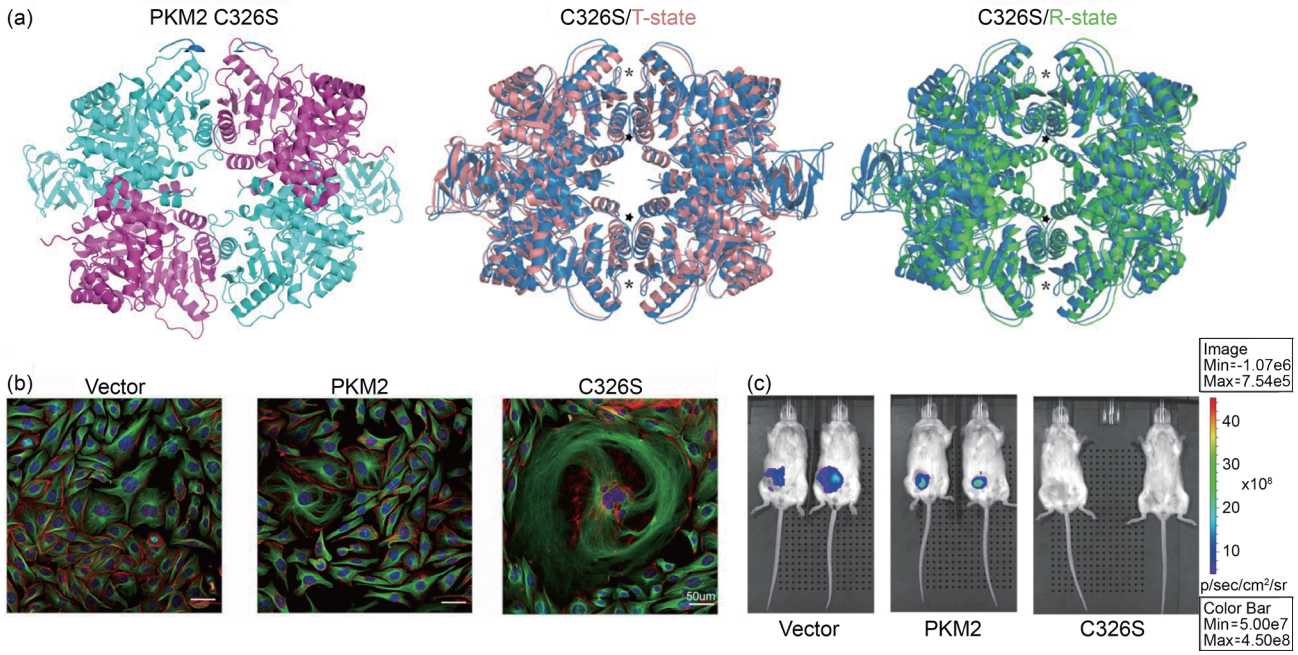

Fig. 2: (a) Structural analysis comparison of the crystal structure of PKM2 C326S mutant with both T-state (phenylalanine-bound) and R-state (FBP/serine-bound) conformations, highlighting key regions: the effector loop and α14-α15 regions, which are indicated by asterisk and star, respectively. The overlaid structures are color-coded as C326S mutant in blue, T-state in pink, and R-state in green to allow for direct comparison of conformational differences between these states. (b) Immunostaining of MDA-MB-231 cells expressing vector, PKM2, or $PKM2^{C326S}$, showing actin (red), α-tubulin (green), and nuclei (DAPI, blue). Images are displayed at 50 μm scale. (c) Bioluminescence imaging of tumor growth in mouse mammary fat pads at week 7 post-implantation of MDA-MB-231 cells expressing different PKM2 variants (vector, wild-type, or C326S). [Reproduced from Ref. 2]

The researchers observed significant negative effects on cancer cell division and proliferation in cells expressing $PKM2^{C326S}$. These cells showed an increased percentage of polyploidy cells and a higher frequency of giant multinucleated cells (**Fig. 2(b)**). Time-lapse microscopy revealed a 1.5-fold increase in cytokinesis failure in $PKM2^{C326S}$-expressing cells. Furthermore, $PKM2^{C326S}$ failed to interact with the spindle checkpoint protein BUb3, and cells showed reduced proliferation rates. Similar effects were observed in cells with H_2S depletion, supporting the specific role of sulfhydration in these processes.

Perhaps most significantly, in a mouse xenograft model, tumor growth was completely suppressed in the $PKM2^{C326S}$ group (**Fig. 2(c)**). While there were no significant differences in mouse body weight between groups, there was a dramatic reduction in tumor bioluminescence signals in the $PKM2^{C326S}$ group, indicating strong anti-tumor effects.

These comprehensive results demonstrate that H_2S-mediated sulfhydration of PKM2 at C326 is a crucial mechanism regulating cancer cell metabolism. Blocking this modification through the $PKM2^{C326S}$ mutation leads to the stabilization of PKM2 tetramers, enhanced oxidative phosphorylation, reduced nuclear translocation and transcriptional activity, impaired cell division, and suppressed tumor growth. These findings suggest that targeting PKM2 sulfhydration could be a promising therapeutic approach for cancer treatment, particularly by rewiring glucose metabolism from aerobic glycolysis to oxidative phosphorylation. The study provides both mechanistic insights into cancer metabolism and potential therapeutic strategies for future drug development.
(Reported by Chun-Hsiang Huang)

This report features the work of Hui-Chun Cheng and her collaborators published in Nat. Commun. ***15****, 7463 (2024).*

TPS 07A Micro-focus Protein Crystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

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Structure of the Prc–NlpI–MepS Complex: Elucidating the Regulatory Mechanism of Bacterial Cell Walls

This study reveals how the adaptor protein NlpI regulates the activity and cellular levels of the cell wall endopeptidase MepS, which facilitates peptidoglycan remodeling and maintains cell wall integrity during bacterial growth and development.

Peptidoglycan (PG) is vital for protecting bacterial cells from osmotic pressure. It consists of linear glycan strands of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM), linked to short peptide chains. The main cross-linking of the short peptide chains is the 4–3 linkage between D-Ala and meso-diaminopimelic acid (DAP), forming a net-like structure that prevents osmotic rupture. During cell growth, the net-like structure must be cleaved to incorporate new PG strands, a process facilitated by several endopeptidases, including MepS, MepM, and MepH. In *Escherichia coli* (*E. coli*), these three endopeptidases are essential for cell wall expansion and their absence leads to abnormal cell shapes and lysis.

NlpI is an outer membrane-anchored lipoprotein found in Gram-negative bacteria (*e.g.*, *E. coli*) and plays multiple roles in cell division, cell wall metabolism, virulence, and host interactions. It interacts with various hydrolases and associates with the PG synthesis machinery, influencing the stability of cell envelope components. As an adaptor protein, NlpI can bind to three endopeptidases—MepS, MepM, and PBP4—and facilitates the formation of trimeric complexes (*e.g.*, MepS-NlpI-PBP4). In addition, NlpI helps localize these enzymes, connecting PG hydrolysis to expansion. Reconstitution experiments show that NlpI organizes PG multienzyme complexes, suggesting it aids in integrating hydrolases and synthases during PG expansion.