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Biomedicine COMMENTARY

Engineering bacterial biocatalysts via directed evolution to deprive cancer cells of cyst(e)ine

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Cyst(e)ine is essential for antioxidant response and cyst(e)ine deprivation agents have emerged as promising cancer therapeutics. In a research article recently published in Cell Metabolism, Wang et al developed cyst(e)ine catabolizing Escherichia coli through directed evolution for targeted cyst(e)ine deprivation cancer therapy. These bacteria are able to specifically enrich in the tumor microenvironment to deplete cyst(e)ine and induce ferroptosis in cancer cells. In this commentary, we discussed the importance of their findings, the promise and challenge of engineering bacteria for metabolic cancer therapies.

It has long been proposed that bacteria could be engineered into programmable "robot factories" for targeted cancer therapies.[1] Previous studies have shown anti-tumor activities of several genera of bacteria in

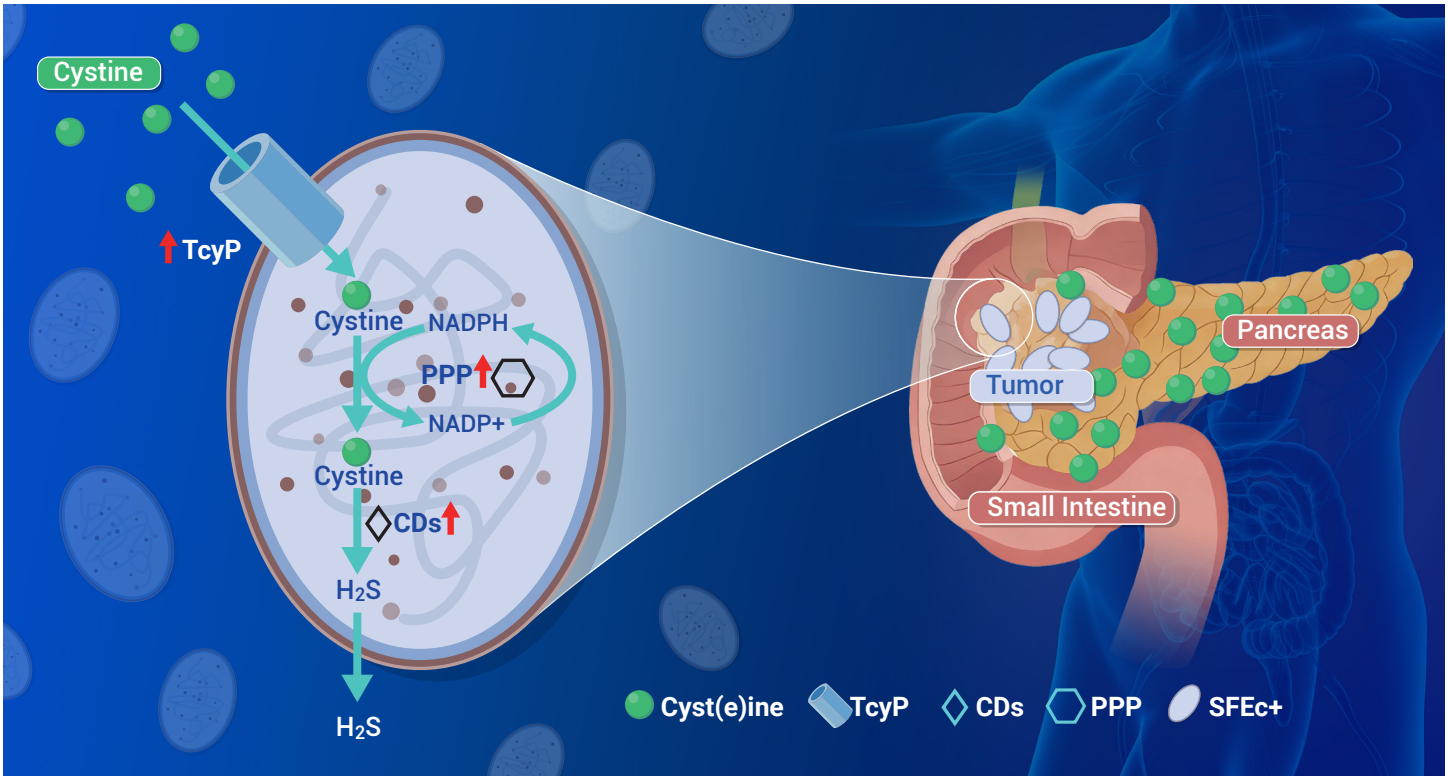

Figure 1. SFEc+ bacteria selectively target tumor tissues for cyst(e)ine deprivation Left, schematic illustration showing that SFEc+ E. coli upregulates cystine transporter TcyP, cysteine catabolizing isozyme CDs and pentose phosphate pathway (PPP) to promote the catabolism and depletion of environmental cyst(e)ine. Cystine uptake by the bacteria was first reduced into cysteine while consuming NADPH, which is regenerated by the PPP. CDs use cysteine as substrate to produce H2S. Right, colonization of tumor tissues by SFEc+ selectively deprived the tumor cells of cystine without affecting normal pancreas tissues.

preclinical animal models, and oncolytic responses were observed in earlier human trials[1]. When systemically administered at a low dose, live bacteria can be rapidly cleared by macrophages and neutrophils in the liver and spleen.[1] However, the hypoxic and immunosuppressive tumor microenvironment (TME) provides a unique niche for tumor-targeting bacteria to survive and thrive.[2] In a study recently published in Cell Metabolism, Wang et al. demonstrated that Escherichia coli (E. coli) can be engineered into cyst(e)ine-catabolizing biohybrids that selectively home to the TME to induce ferroptotic tumor cell death.[3]

Cyst(e)ine is a conditionally essential amino acid required for the synthesis of glutathione, a tripeptide necessary for the antioxidant response and resistance to ferroptosis. In recent years, cyst(e)ine-deprivation agents have attracted much attention due to their ability to induce ferroptosis in drug-resistant and metastatic cancer cells. The epithelial-mesenchymal transition (EMT) program, which promotes therapy resistance and tumor metastasis, also enhances the synthesis of polyunsaturated fatty acid (PUFA)-containing lipids and therefore increases the susceptibility of cancer cells to ferroptosis inducers. Several FDA-approved drugs, including sulfasalazine and sorafenib, have been shown to inhibit the cystine transporter system xc^-6. More selective and potent system xc^- inhibitors, such as imidazole ketone erastin (IKE), have also been developed7. Although these inhibitors can block system xc^--mediated cystine uptake and inhibit tumor growth in animal models, cysteine can still be imported by cancer cells through the L-amino acid transporter 1 (LAT1). The development of optimized L-cyst(e)inase enables the systemic depletion of both cystine and cysteine and has markedly improved the efficacy of cyst(e)ine-deprivation therapy. Wang et al. took a step further by developing $SFEc^+$, an L-cyst(e)ine-catabolizing E. coli strain that allows for selective depletion of cyst(e)ine in the TME.[3]

E. coli can uptake cystine through its cystine transporter TcyP and catabolize excess cysteine by producing H_2S. Wang et al. employed an iterative directed-evolution approach to enhance both cystine uptake and H_2S production by E. coli. First, bacteria were cultured in thiol-free media to enhance cystine

uptake. Next, $PbAc_2$ plates were used to select bacterial colonies with the strongest H_2S production activity. After thousands of generations of amplification in thiol-free medium and three rounds of $PbAc_2$ selection, they obtained SFEC$^+$, an E. coli strain with a 36.5-fold higher cystine uptake rate and 23-fold higher cysteine-catabolizing activity. Importantly, SFEC$^+$ was able to completely deplete cyst(e)ine in the culture within 7.5 hours.

A striking feature of the dual-selection scheme is its ability to identify and enrich E. coli strains that retain high H_2S production activity while adapting to thiol-free growth conditions (Figure 1). As one might imagine, catabolizing cysteine to produce H_2S would be wasteful and detrimental to the fitness of bacteria when cysteine is scarce. Therefore, after multiple generations of adaptation, bacteria might weaken H_2S production while enhancing cystine uptake. The dual-selection strategy employed by Wang et al. allowed for the selection of bacteria with enhancements in both cystine uptake and catabolism. Simultaneous activation of cyst(e)ine uptake and catabolism is critical for bacteria to rapidly and efficiently deplete environmental cyst(e)ine (Figure 1). E. coli colonies that adapted to thiol-free culture but exhibited low H_2S production activity had significantly lower cyst(e)ine depletion capacity, further supporting the importance of the dual-selection strategy.

Transcriptomic profiling of SFEC$^+$ by Wang et al. revealed profound reprogramming of multiple metabolic pathways during the directed-evolution engineering of E. coli. In addition to the upregulation of genes involved in cyst(e)ine uptake and catabolism, metabolic pathways that produce NADPH—such as the pentose phosphate pathway (PPP) and the tricarboxylic acid (TCA) cycle—were markedly upregulated. NADPH levels were also significantly higher in SFEC$^+$ compared to parental E. coli. This upregulation of NADPH-producing pathways may be critical for SFEC$^+$ to catabolize cystine, as H_2S-producing cysteine desulfurases (CDs) can only use cysteine as a substrate, and the reduction of cystine to cysteine consumes NADPH (Figure 1). Interestingly, pancreatic cancer cells adapted to low-cystine media also upregulate PPP and NADPH production.[4] NADPH is a critical reducing equivalent for the antioxidant response, and its levels are a robust predictor of ferroptosis resistance in mammalian cells. Ferroptosis-like cell death has even been reported in bacteria. Therefore, increased NADPH production in SFEC$^+$ bacteria may also be essential for their survival under thiol-free conditions.

To further improve cystine-deprivation capacity, Wang et al. conjugated SFEC$^+$ with liposomes loaded with DMXAA (DL), an anti-vascular agent, to generate the DL@SFEC$^+$ biohybrid. When added to cancer cell cultures, both DL@SFEC$^+$ and SFEC$^+$ induced cystine depletion, lipid peroxidation, and ferroptosis, consistent with their robust cyst(e)ine-catabolizing activities. The DL@SFEC$^+$ biohybrid can simultaneously disrupt nutrient supplies and deplete cyst(e)ine in the tumor microenvironment (TME). An additional benefit of conjugating DL with SFEC$^+$ is that the tumor tropism of SFEC$^+$ increases the local concentration of DL in the TME. Furthermore, disruption of the tumor vasculature facilitates infiltration and colonization of the TME by SFEC$^+$. Indeed, intravenous injection of DL@SFEC$^+$ induced more severe vascular rupture and better bacterial colonization than co-injection of unconjugated DL plus SFEC$^+$.

Wang et al. used three different preclinical models of colorectal and pancreatic cancer to evaluate the anti-tumor activities of DL@SFEC$^+$. In all three models, SFEC$^+$ achieved impressive inhibition of tumor growth and oncogenesis, and the anti-tumor effects were further enhanced by DL@SFEC$^+$ conjugates. Analysis of mice treated with the biohybrid confirmed near-complete depletion of tumor cysteine five days after DL@SFEC$^+$ administration.

A potential concern with live bacteria-based therapy is the risk of infection. However, a comprehensive analysis by Wang et al. provided compelling evidence supporting the excellent biosafety profile of DL@SFEC$^+$. In mice administered with DL@SFEC$^+$, the live bacteria were cleared from all major organs within 24–96 hours. Blood biochemistry, complete blood count, and pathological analyses of harvested major organs indicated no detectable adverse effects.

Interestingly, despite time-dependent clearance of SFEC$^+$ from major organs, the number of intratumor bacteria increased over time, indicating expansion of DL@SFEC$^+$ within the TME. Importantly, bacteria isolated from tumors retained H_2S-producing activity. This is consistent with in vitro data showing that SFEC$^+$ maintains stable cyst(e)ine-catabolizing abilities after 80 generations of culture under physiological cyst(e)ine conditions. Thus, Wang et al. convincingly demonstrated that DL@SFEC$^+$ can selectively target the TME to continuously deplete cyst(e)ine and induce ferroptosis.

The study by Wang et al. shows that directed-evolution approaches can be used to engineer live bacteria into biocatalysts that starve tumor cells of nutrients. In addition to cyst(e)ine, cancer cells are also addicted to other amino acids such as glutamine and serine. It is possible that a similar strategy could be used to deplete other essential nutrients in the TME. Bacteria-based metabolic therapies offer several advantages over conventional enzyme- or inhibitor-based approaches. First, the tumor tropism and immunosuppressive nature of the TME allow nutrient-catabolizing bacteria to selectively deplete essential nutrients within tumors (Figure 1), potentially enabling more extreme nutrient restriction while avoiding toxicity in normal tissues. Second, bacteria can achieve relatively stable, long-term nutrient depletion in tumors—an advantage over conventional therapies. Third, by conjugating bacteria with liposomes carrying additional cancer therapeutics, they can serve as delivery vehicles to combine metabolic and conventional therapies.

Although the DL@SFEC$^+$ biocatalyst developed by Wang et al. showed impressive short-term anti-tumor effects, the long-term outcomes remain to be determined. A major challenge in metabolic therapy is the ability of cancer cells to rewire metabolic pathways in response to nutrient deprivation. For example, pancreatic cancer cells may increase glucose metabolism, PPP flux, and NADPH production to survive chronic cystine limitation.[5] Such metabolic adaptation can promote ferroptosis resistance, tumor growth, and metastasis. It will be interesting to see whether resistant tumor cells emerge in DL@SFEC$^+$-treated animals, for example, by monitoring upregulation of PPP enzymes such as G6PD and PGD in tumor biopsies from DL@SFEC$^+$-treated mice. A potential complication from systemic administration of DL@SFEC$^+$ is potential infection risk. Although the authors performed extensive analysis to show the clearing of bacteria in most major organs, the weaken immune system among cancer patients could significantly increase infection risk. In addition, human is more sensitive to bacterial endotoxin than mouse, which could limit the maximum dose of systemic administration of DL@SFEC$^+$.

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AUTHOR CONTRIBUTIONS

Y.L. and S.Y. wrote the manuscript. Q.L. organized related articles. M.T. drawn the picture of the commentary. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.