Cloaking bacteria through tunable gene circuits to treat cancer

Microbiome serves various functional roles in human wellbeing. This has resulted in a convergence of interests in the increased use of live bacteria to treat diseases. Microbes can colonize niches in the gastrointestinal tract, mouth, skin, lung, and malignancies and deliver therapies locally because they can be developed as intelligent living medicines that perceive and respond to their surroundings. However, host toxicity from live bacteria has been found to restrict tolerable dosage and efficacy, leading in some instances to the cancellation of clinical trials. Furthermore, unlike traditional drug carriers, bacteria's unique ability to constantly multiply, translocate, and deliver therapeutic payloads in cancerous tissue needs powerful and temporal regulation of bacterial pharmacokinetics *in vivo*.

Now, the researchers at Columbia University's School of Engineering and Applied Science have developed a 'cloaking' system that temporarily conceals therapeutic bacteria from immune systems, allowing them to deliver drugs to tumours and kill cancer cells more efficiently in mice. By changing the DNA of the microbes, they were able to programme gene circuits that control the bacterial surface, resulting in the formation of a molecular 'cloak' that encapsulates the bacteria. The researchers concentrated on capsular polysaccharides (CAP), a sugar polymer covering bacterial surfaces for the study[1]. Numerous bacteria in nature utilise CAP to shield themselves from threats, including immune systems. Because of its excellent clinical characteristics and great survivability in human blood, the researchers chose a probiotic *Escherichia coli* Nissle 1917 strain as the bacterium carrier. With CAP, these bacteria may resist immune attack for a short time; without CAP, they lose their encapsulation protection and are removed from the body. As a result, the researchers opted to construct an effective on/off switch by developing a novel CAP system called inducible CAP, or iCAP. They operate the iCAP system by providing an external cue, a tiny chemical known as IPTG, allowing programmable and dynamic changes to the *E. coli* cell surface. Because iCAP alters bacterial interactions with immune systems (such as blood clearance and phagocytosis) in a regulated manner, the researchers found that by changing the quantity of IPTG delivered to the iCAP *E. coli*, they could control the duration for which bacteria can dwell in human blood.

While utilizing bacteria for therapy offers a novel, alternative method to treat a wide range of malignancies, there are several barriers to overcome; most notably their toxicity. These bacteria are live and can multiply in the body, unlike conventional medications. They are also identified as foreign and hazardous agents by the body's immune systems, resulting in either a strong inflammatory response (too many bacteria implies high toxicity owing to over-inflammation) or swift bacteria removal (too little bacteria means little therapeutic effectiveness). These toxicities have been found to be a key issue in clinical trials, restricting the amount of bacteria that researchers can dose and reducing effectiveness. Due to significant toxicity, several trials had to be abandoned.

The ideal bacterium should be able to enter the body without being detected by the immune system and swiftly reach the tumour. And, once within the tumour, they must be cleared in other regions of the body to reduce toxicity. The research team utilised mouse tumour models to show that by using iCAP, they could boost the maximum tolerable dosage of bacteria by tenfold. They encapsulated the *E. coli* strain so that it may avoid detection by the immune system and reach the tumour. Because scientists did not provide IPTG to the *E. coli* iCAP, it lost its encapsulation over time and was easier to clear in other body areas, thus reducing toxicity. To assess effectiveness, the researchers modified *E. coli* iCAP to create an antitumor toxin and found that it reduced tumour development in colorectal and breast cancer mouse models more than the control group without the iCAP system. The researchers also showed that bacterial movement throughout the body could be controlled. Earlier studies reported that as tumours develop, low bacteria levels seep out. The researchers used iCAP to demonstrate that they can regulate bacterial leaking from tumours as well as their translocation to other tumours in this new study. They injected *E. coli* iCAP into one tumour, gave the mice IPTG-laced water, activated iCAP inside a tumour, and noticed *E. coli* iCAP leak out and move to non-injected tumours.

Bacillus Calmette-Guerin, or BCG, is the only FDA-approved bacteria-based cancer treatment for non-muscle invasive bladder cancer, and it was developed in the late 1970s. It works by prompting an immunological response to cancer and directly destroying cancer cells. Some companies are developing oral microbiome treatments with the hope of curing cancer by modifying the gut microbiome, while others are concentrating on utilizing modified bacteria to deliver protein payloads to tumours selectively. Currently, the research team is investigating a variety of study topics. There are about 80 distinct forms of CAP for *E. coli* alone and even more for other bacteria species that may be developed using similar methods. Furthermore, CAP is not the only molecule found on the surface of bacteria, and other surface molecules could be regulated similarly. Moreover, while in this case, iCAP is regulated by an externally supplied IPTG, alternative control systems such as biosensors might be utilized to modulate surface features of therapeutic bacteria autonomously. The team, also affiliated with Columbia's Herbert Irving Comprehensive Cancer Center and Data Science Institute, cites clinical translation as the next key hurdle they would like to overcome. While a plethora of laboratory research reveals different approaches to engineer microorganisms, applying these strong therapies to a complex animal or human body is exceptionally challenging. The researchers have provided proof of concept in mouse models, but because humans are 250 times more susceptible to bacterial endotoxins than mice, they anticipate that the findings will have a greater impact on human patients than on mice. They further said that bacterial cancer treatment has distinct benefits over traditional medication therapy, such as effective tumour tissue targeting and programmed drug release. The possibility of toxicity has hampered its full potential. This study's cloaking strategy may address this key issue.

1. Harimoto, T. *et al.*, *Nat. Biotechnol.*, 2022; doi:10.1038/s41587-022-01244-y

Arjun R. Krishnan
e-mail: arjunkriz92@gmail.com