MICROBIAL ENGINEERING

Bacterial couriers as cancer vaccines

Flagellated bacteria coated with antigen-adsorbing nanoparticles and injected into irradiated tumours elicit systemic antitumour immune responses by transporting tumour antigens towards the tumour periphery, where they are taken up by functional antigen-presenting cells.

Andrew Redenti, Jaeseung Hahn and Tal Danino

Cancer vaccines are designed to enhance immune responses towards tumour antigens. To deliver tumour antigens to lymphoid tissues, these vaccines employ a range of vector types, such as synthetic peptides, live-attenuated bacteria, autologous immune cells and mRNA nanoparticles[1–3]. Radiotherapy and chemotherapy, which induce the release of antigens on tumour-cell death, can also increase the exposure of tumour antigens to the immune system[4]. Regardless of the type of vector and whether the release of tumour antigens is stimulated, adaptive antitumour immunity involves antigen-presenting cells (APCs) taking up, processing and presenting the antigens to T cells. However, the immunosuppressive tumour microenvironment can render tumour-resident APCs dysfunctional and unable to exert control over the tumour. Writing in *Nature Biomedical Engineering*, Jinhui Wu, Yiqiao Hu and co-authors now show that systemic antitumour immunity can be elicited in irradiated immunosuppressive tumours by injecting motile bacteria coated with antigen-adsorbing nanoparticles into the tumours[5]. The bacteria transport the tumour antigens to functional dendritic

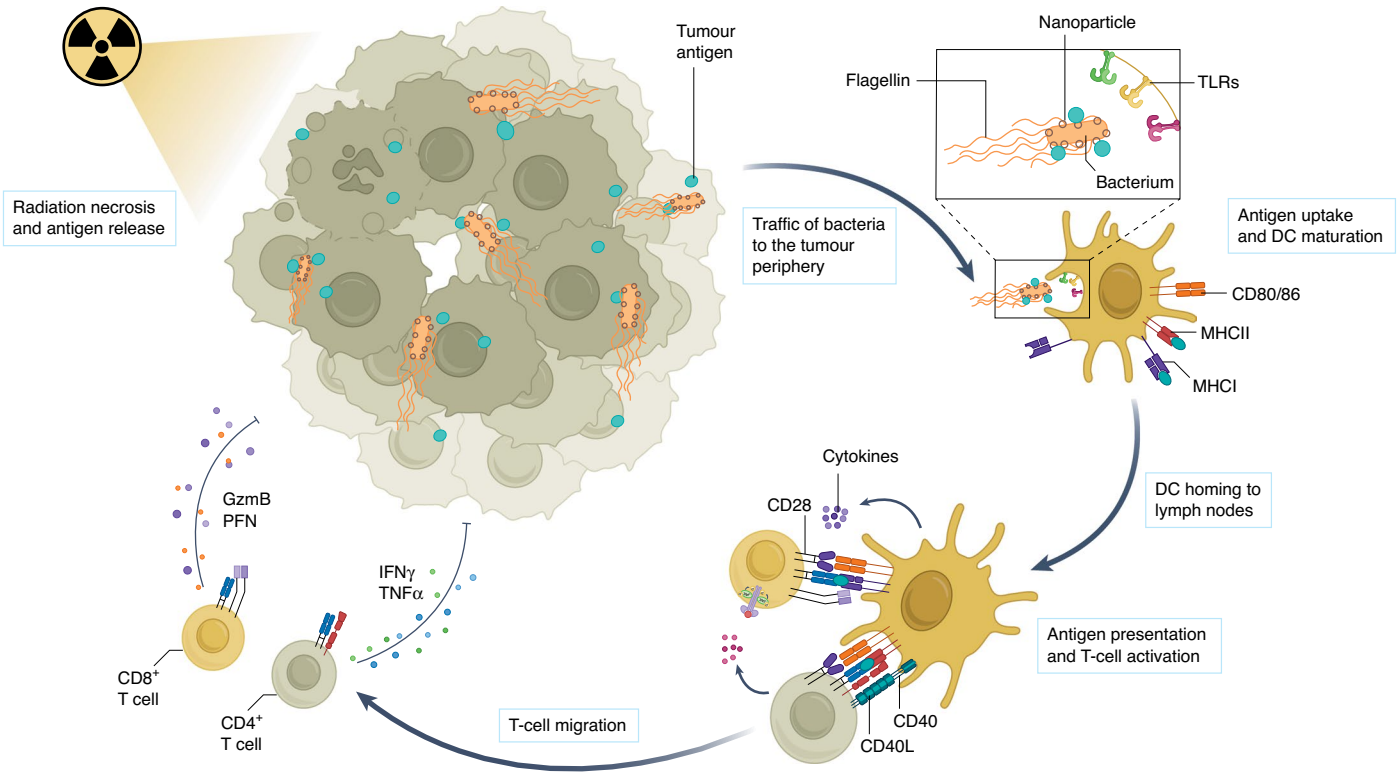

Fig. 1 | Motile bacteria coated with antigen-adsorbing nanoparticles trigger antitumour immunity following radiotherapy. Flagellated bacteria coated with cationic nanoparticles capture negatively charged tumour antigens released after radiation-induced tumour-cell death and travel towards the periphery of the tumour. Functional DCs at the tumour periphery phagocytose the bacteria, undergo maturation mediated by TLRs, and migrate to tumour-draining lymph nodes via lymphatic vessels. In the lymph nodes, mature DCs present tumour antigens to naive T cells, which leads to T-cell activation. The activated tumour-antigen-specific T cells traffic through the vasculature to the tumour, where they exert their effector function. CD, cluster of differentiation; GzmB, granzyme B; IFNγ, interferon-γ; MHCI and MHCII, major histocompatibility factors; PFN, perforin.

cells (DCs) — a subset of APCs — at the tumour periphery.

Wu and co-authors used an attenuated strain of *Salmonella* Typhimurium (VNP20009) coated with positively charged polyamidoamine dendrimer nanoparticles, which can bind negatively charged antigens through electrostatic interactions. VNP20009 contains chromosomal deletions for the genes *purI* and *msbB*. This *purI*$^-$ bacterial strain is auxotrophic for adenine, and the deletion of the *msbB* gene makes the bacterium less immunogenic via a modified lipopolysaccharide that reduces tumour necrosis factor-α (TNFα)-mediated toxicity[6]. The authors first observed that nanoparticle-coated VNP20009, because it is motile and able to colonize tumours, captures and disseminates ovalbumin (OVA) — a negatively charged model antigen — throughout agar and plated cancer spheroids. Then, by comparing nanoparticle-coated VNP20009 to uncoated VNP20009 and to nanoparticle-coated yet heat-killed (and, hence, immotile) VNP20009, the authors confirmed that the antigen-transporting effects of the bacteria require that they be motile and that they carry the antigen-capturing nanoparticles on their surface. The authors also show that the antigen-capturing bacteria can transport OVA to DCs across a membrane and that the DCs can then present OVA-derived antigens (OVA–peptide) on major histocompatibility complex (MHC) molecules on the cell's surface. Hence, DCs can take up OVA-adsorbed bacteria, properly process the relevant OVA epitopes, and present them on MHCI molecules.

Cancer cells employ a variety of mechanisms to evade antitumour immunity, including the physical exclusion of immune cells, the expression of immunoinhibitory molecules, and alterations to each step of the antigen-processing and antigen-presentation pathways[7–9]. Many strategies have been devised to increase the number of intratumoural DCs and to augment their function; in many cases, however, the immunosuppressive tumour microenvironment continues to substantially hinder antitumour immunity. In fact, in a murine model of melanoma expressing OVA (B16-OVA), Wu and colleagues show (via immunofluorescence) that CD103$^+$CD11c$^+$ DCs (an APC subpopulation critical for the stimulation of cytotoxic T-cell responses) are excluded at the periphery of the tumours. This implies that these DCs cannot enter the tumour to retrieve antigens, even after radiotherapy-triggered antigen release.

Wu and co-authors' therapeutic strategy takes advantage of the motility of flagellated bacteria to get tumour antigens transported out of irradiated tumours and to functional DCs at the tumour periphery. This significantly increased the populations of OVA–peptide-presenting DCs. Moreover, the authors show that the bacteria also act as strong stimulants of the activation of antigen-specific immunity (Fig. 1). Intricate combinations of receptors (such as the Toll-like receptors (TLRs), which recognize a range of viral and microbial products) allow DCs to sense their internal and external environments. Immature DCs patrol tissues, sample antigens, and begin the process of maturation after encountering microbial ligands or an inflammatory milieu of cytokines. During this process, DCs migrate to lymph nodes to activate adaptive immunity[10]. Bacterial components potently stimulate this maturation process (microbial lipopeptides stimulate TLR2; lipopolysaccharides, TLR4; flagellin, TLR5; and unmethylated oligodeoxynucleotides, TLR9) and can work individually and in synergy to instruct DCs and thus T-cell fates[11,12]. The authors observed that intratumoural injection of the antigen-capturing bacteria caused the upregulation of the DC-maturation markers CD80 and CD86 in tumour-draining lymph nodes, and that this led to increased numbers of OVA–peptide-specific T cells in B16-OVA tumours. Overall, the injection of the antigen-capturing bacteria after radiotherapy aided the delivery of tumour antigens to DCs and prompted DC maturation and the migration of these cells to tumour-draining lymph nodes. This resulted in the enhanced activation and migration of naive CD8$^+$ T cells.

Because model-antigen systems such as B16-OVA may lack sufficient physiological relevance, Wu and co-authors investigated how native tumour-mutant proteins interacted with the antigen-capturing bacteria in a murine model of colon carcinoma by incubating the bacteria with tumour-cell lysates obtained after the application of radiotherapy. They show via mass spectrometry that the bacteria captured a vast array of previously identified tumour-mutant proteins. This suggests that the antigen-capturing bacteria can be used as in situ cancer vaccines without the need for the pre-identification of antigens (this can be technically challenging, and the antigens are often patient-specific[13]). In addition, by using mice bearing two tumours, one on each side of the abdomen, the authors show that multiple rounds of radiotherapy combined with intratumoural injection of antigen-capturing bacteria in one of the tumours resulted in superior tumour control (when compared with the injection of bacteria alone, the application of radiotherapy alone, or the combination of radiotherapy and bacteria lacking antigen-adsorbing nanoparticles), with higher numbers of CD8$^+$ and CD4$^+$ T cells. The tumours that did not receive therapy (radiotherapy and bacteria) directly grew more slowly, indicating the presence of systemic antitumour immunity via the abscopal effect.

Immunosuppression via the immune-checkpoint protein programmed-death ligand-1 (PD-L1) can inhibit the proliferation and effector function of tumour-specific immune cells[14]. By combining tumour irradiation and intratumoural injection of antigen-capturing bacteria with the intraperitoneal administration of a PD-L1-blocking antibody, Wu and colleagues observed greater antitumour responses than those achieved with the combination of radiotherapy and PD-L1 blockade in the absence of bacteria. The bacteria increased the number of effector CD8$^+$ and CD4$^+$ T cells and decreased the amount of regulatory T cells in the tumours, suggesting that the injection of the bacteria into the tumours synergizes with checkpoint blockade in the presence of radiotherapy.

Wu and co-authors' findings suggest that exploring the contributions of bacterial motility and bacterial growth to antigen transport could help to guide the development of better strategies for the transportation of tumour antigens. Investigating the influence of the nanoparticles' surface charge on the observed antitumour responses, especially in an antigen-agnostic context, would be informative as to whether there are other mechanisms at play, such as increased bacterial uptake by immune cells (through electrostatic interactions). Intravenous delivery of the bacteria would be desirable as it would enhance their applicability to inaccessible tumours or to tumours of unknown location. However, systemic delivery of the bacteria may lead to the adsorption of non-tumour molecular species, and replication of the bacteria could result in the shedding of the nanoparticles. It would also be fitting to investigate whether the combination of immunostimulant bacteria and checkpoint blockade in the absence of radiotherapy elicits substantial antitumour immunity. Ultimately, it should be possible to engineer microbes (or even chemically fuelled or externally controlled micromotors and nanomotors[15]) that boost immunogenic cell death and facilitate the appropriate localization of tumour antigens to enhance antitumour immunity.

All in all, Wu and co-authors' work suggests that, when combined with

radiotherapy, antigen-capturing flagellate bacteria can act as potent in situ cancer vaccines by facilitating antigen uptake by functional DCs at the tumour periphery, thus enhancing T-cell activation. Attenuated or tailored bacterial strains with low virulence and high antibiotic sensitivity that retain their immunostimulatory function and tumour specificity may thus be suitable candidates for the design of new cancer vaccines. ❐

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Competing interests

The authors declare no competing interests.