



Bacteria in cancer therapy

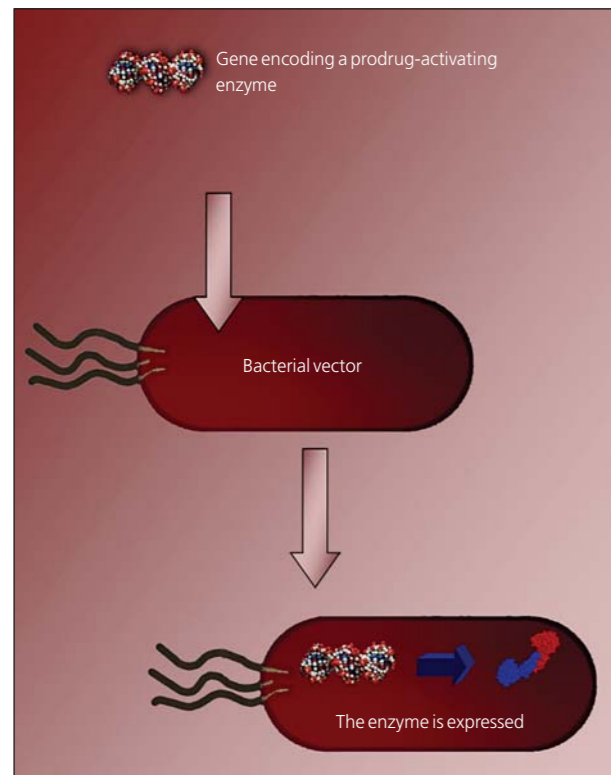
Bacteria may well have an exciting role to play in the treatment of cancer, as **Caroline Springer, Panos Lehouritis** and **Richard Marais** explain.

The observation that bacteria could be used as anti-cancer agents dates back 150 years. The German physicians W. Busch and F. Fehleisen separately observed that certain types of cancers regressed following accidental erysipelas (*Streptococcus pyogenes*) infections that occurred whilst patients were hospitalized. Independently, the American William Coley noticed that one of his patients suffering from neck cancer began to recover following an infection with erysipelas. Coley was so excited with this finding that he devoted his career to researching the use of bacteria for cancer therapy. Sadly, none succeeded in finding a cure for cancer due to the toxicity complications arising from these types of therapies. However, their findings provided the grounds for today's advances in this field.

Bacteria as cancer therapy

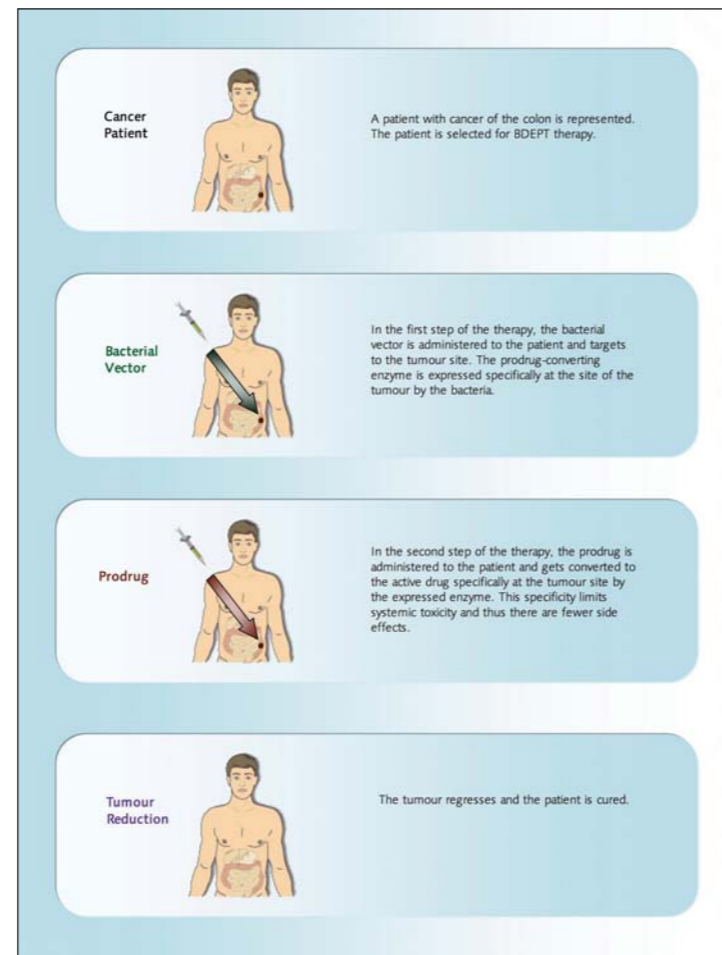
Several decades after Coley's work, interest re-emerged in the use of bacteria to treat solid tumours. Experiments showed that pathogenic species of the anaerobic clostridia were able to proliferate preferentially within the necrotic (anaerobic) regions of tumours in animals compared to normal tissues. This resulted in tumour regression but was accompanied by acute toxicity and most animals became ill or died. Researchers then changed to a non-pathogenic strain of

◀ A female patient receiving cancer chemotherapy treatment.
Will & Deni McIntyre / Science Photo Library



▲ Fig. 1. Construction of a BDEPT vector. A gene encoding a prodrug-converting enzyme is used to arm a bacterial gene therapy vector that has the ability to localize to tumour sites. This gene expresses the therapeutic enzyme inside the bacterial cell (The expression of the enzyme Carboxypeptidase G2 is shown as an example). C.J. Springer

► Fig. 2. A schematic of the BDEPT principle. C.J. Springer



Clostridium such as 'M55', showing that it was able to colonize anaerobic parts of the tumour following intravenous administration and this therapy progressed to human clinical trials. Ironically, this safer strain did not produce significant tumour regression so the trials were stopped.

Recently, researchers have screened a number of anaerobic bacterial species (bifidobacteria, lactobacilli and pathogenic clostridia) for their ability to accumulate in experimental tumours in animals. *Clostridium novyi* was found to be the most successful candidate to demonstrate significant anti-tumour effects, but these experiments too culminated in death. A gene coding for a lethal toxin was subsequently deleted from the genome of *C. novyi*, resulting in its attenuation. Therapy experiments using the modified bacteria in combination with classical chemotherapy demonstrated phenomenal results and attracted media attention. Unfortunately, even after attenuation, the therapy was not devoid of animal deaths. *C. novyi* has now been investigated in conjunction with radiotherapy, radio-immunotherapy, and further chemotherapy in experimental tumour models

demonstrating some success. Recent research into its mechanism of action revealed that it was capable of stimulating an immune response to attack and destroy the tumour. The level of inflammation induced was responsible for the animal deaths. A similar immunostimulatory mechanism of action has also been attributed to *Bacillus Calmette–Guerin* (BCG), the most successful bacterial agent so far, used specifically for the treatment of superficial bladder cancer.

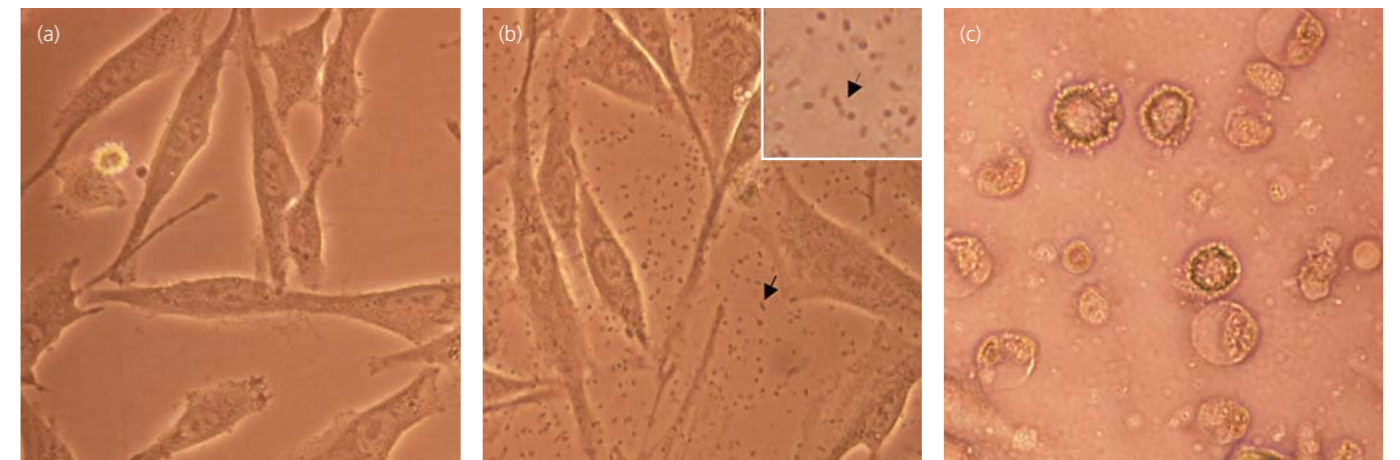
A derivative strain of *Salmonella typhimurium* has now been developed for use in cancer treatment. Deletion of two of its genes – *msbB* and *purI* – resulted in its complete attenuation (by preventing toxic shock in animal hosts) and dependence on external sources of purine for survival. This dependence rendered the organism incapable of replicating in normal tissue such as the liver or spleen, but it was still able to grow in tumours where purine is available. This vector showed long-lasting efficacy against a broad range of experimental tumours and was even able to target metastatic lesions. Its mechanisms of tumour suppression are not entirely understood but are quite dis-

tinct from the previously described bacteria and seem to be dependent on specific genes associated with pathogenicity rather than the stimulation of the immune system.

Research in this field is growing and new strains of bacteria are being investigated as anticancer agents: *Salmonella choleraesuis*, *Vibrio cholerae*, *Listeria monocytogenes* and even *Escherichia coli* have all been shown to replicate within tumours.

Bacterially Directed Enzyme Prodrug Therapy (BDEPT)

As discussed, the major problem with using bacteria as anti-cancer agents is their toxicity at the dose required for therapeutic efficacy. Reducing the dose whilst significantly reducing the toxicity results in diminished efficacy. One approach to overcome this limitation has been to use Gene-Directed Enzyme Prodrug Therapy (GDEPT), a two-step alternative to standard chemotherapy aimed at delivering the therapeutic agent to the site of the tumour and thus limiting its unacceptable side effects. This involves 'arming' bacteria with genes encoding foreign enzymes that have the ability to convert non-toxic



▲ Fig. 3. A *Salmonella* vector expressing CPG2 is a potent anti-tumour agent. (a) Light microscopy showing tumour cells grown *in vitro*. (b) Tumour cells incubated with *Salmonella* expressing CPG2 (an individual bacterial cell is shown by the arrow). The tumour cells are not affected by the presence of bacteria. (c) In the presence of prodrug, the CPG2 enzyme, expressed by *Salmonella*, converts the prodrug into a cytotoxic agent, resulting in induction of tumour cell death, which is evident by the significant change in the morphology of the tumour cells. C.J. Springer

As more developments occur, it is hoped that BDEPT, an exciting new field in cancer therapy, will live up to its promise.

prodrugs to cytotoxic drugs (Fig. 1). A typical therapy regime involves intravenous administrations in two separate steps (Fig. 2). In the first step, the bacteria are administered (at safe levels) that target the tumour where they proliferate and express the therapeutic enzyme. In the second step, once the expression of the enzyme is optimal, a non-toxic prodrug is administered which is converted to the cytotoxic drug at the tumour by the expressed enzyme. This results in tumour cytotoxicity rather than systemic toxicity, leading to tumour cell death and sparing normal tissue (Fig. 2). Because the vector that is delivering the enzyme gene is a bacterium, this is also called Bacterially Directed Enzyme Prodrug Therapy (BDEPT).

Several enzyme/prodrug systems are available. Cytosine deaminase (CD), which converts 5-fluorocytosine (5FC) to 5-fluorouracil (5FU), and nitroreductase (NR), which converts the prodrug CB1954 to a DNA cross-linking agent, have been tested with *Clostridium sporogenes*. Although these combinations can kill tumour cells *in vitro* and deliver high concentrations of enzymes to model tumours, to date, the results *in vivo* have been disappointing.

The *Salmonella* vector has been combined with NR and CD, and success has been observed *in vivo*, prompting a Phase I clinical trial. We have combined *Salmonella* with carboxypeptidase G2 (CPG2), an enzyme that converts a range of mustard prodrugs to DNA cross-linking agents (Fig. 3). CPG2

has been expressed in various compartments within bacterial cells and high levels of activity have been detected in tumours following *in vivo* administration, so research on this system is ongoing. It is worth noting that not all GDEPT systems will be suitable for BDEPT and many barriers will exist. For example, both the prodrug and the activated drug must be able to cross biological membranes, because the prodrug will be activated within bacterial cells and the active drug will then need to enter the tumour cells. This is unlike GDEPT, where the enzyme generally resides within the tumour cells.

BDEPT in humans is still in its infancy and there is much to learn about the targeting mechanisms of the bacteria: do enough bacteria reach the tumour compared to normal tissues; does the prodrug have access to the bacteria; is the prodrug converted to the cytotoxic drug at the tumour; and does the bacterial vector evade our immune systems? As more developments occur, it is hoped that these questions will be addressed and that BDEPT, an exciting new field in cancer therapy, will live up to its promise.

Caroline J. Springer

Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK (t 0208 722 4214; f 0208 722 4046; e caroline.springer@icr.ac.uk)

Panos Lehouritis & Richard Marais

Cancer Research UK Centre for Cell and Molecular Biology, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK (e panos.lehouritis@icr.ac.uk and e richard.marais@icr.ac.uk)