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Bugging Tumors

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Summary

The effects of bacteria on cancer patients have been observed for at least two centuries. Recent studies in animal models of cancer have demonstrated efficacy of both anaerobic bacteria such as *Clostridia* and *Bifidobacteria* and facultative anaerobes such as *Salmonella*. In this issue of *Cancer Discovery*, Flentie et al have identified five *Salmonella* promoters that are specifically stimulated by cancer cells as well as by acid pH, a property of most tumors. One of these promoters (*STM1787*) was linked to a Shiga toxin gene and inserted in a wild-type *Salmonella typhimurium* strain, which showed in vivo antitumor efficacy. Approaches to further improving the efficacy of *S. typhimurium* with the use of tumor-targeting mutations are discussed. Since the barriers to efficacy of standard therapy of cancer appear to be opportunities for bacterial cancer therapy, the future of bacterial therapy of cancer appears bright.

> "…for over 200 years neoplasms have been observed to regress following acute infections, principally streptococcal. If these cases were not too far advanced and the infections were of sufficient severity or duration, the tumors completely disappeared and the patients remained free from recurrence" (1).

Helen Coley Nauts, author of the quote above, was the daughter of William B. Coley, who was an oncologist at what is now known as Sloan-Kettering Memorial Cancer Center in New York in the late 19th and early 20th centuries, Coley infected cancer patients with *Streptococcus pyrogenes* and in later years treated the patients with extracts of the bacteria, which became known as Coley's toxins. Coley had remarkable results with his toxins, but this treatment fell out of favor after Coley's death in 1936 (2).

Recently, there has been intense interest to develop bacterial therapy of cancer using modern methods of bacterial genetics, cancer cell and molecular biology, and in vivo imaging (2, 3). The barriers in tumors for standard therapy to be effective such as hypoxia, acidic pH, disorganized vascular architecture, and dissemination can be opportunities for bacteria to target cancer (3).

In the current issue of Cancer Discovery, Flentie et al. (4) designed a promoterless transposon luciferase reporter to analyze a library containing 7,400 independent *Salmonella* transposon insertion mutants and identified five *Salmonella* promoter specifically activated by cancer cells, as well as by acidic pH often found in tumors.

Flentie et al. (4) utilized the most pH-sensitive promoter (*STM1787*) they identified, to demonstrate the utility of tumor-specific *Salmonella* promoters to drive the Shiga toxin-2 (Stx-2) transgene in a wild-type strain of *S. typhimurium*. *Salmonella* expressing Stx-2 from the *STM1787* promoter had anti-tumor activity *in vivo* as well as in vitro. Intratumoral

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injection of a single high-dose of the wild type *S. typhimurium* strain SB300A1, transformed with P1787-Stx2, resulted in an 80% inhibition of tumor growth five days after treatment. Mice treated with high-dose SB300A1-P1787-Stx2 died, but mice receiving low-dose P1787-Stx2 were healthy for two weeks, at which point they had a significant reduction in tumor size compared to control.

Other approaches to bacterial therapy of cancer have used the anaerobic bacteria, *Bifodobacterium* and *Clostridium*, which replicate in necrotic areas of tumors. These anaerobic bacteria cannot grow in viable tumor tissue which restricts their efficacy. In order for anaerobic bacteria to be effective, they must be used in combination with chemotherapy (3).

S. typhimurium, which is a facultative anaerobe, attenuated with purine and other auxotrophic mutations, has been previously used for cancer therapy (5). *S. typhimurium* with lipid-A-modified (msbB) and purine auxotrophs (purI) did not have toxicity in mice and swine and also had significantly reduced host TNF-a induction (5). In a Phase I clinical trial on metastatic melanoma patients, the *S. typhimurium* strain tested (VNP20009) was attenuated by msbB and purI mutations. VNP20009 was safely administered to patients, but poorly colonized the patients' tumors, perhaps because it is over-attenuated (6).

A new strain of *S. typhimurium*, A1-R, has been developed which has greatly increased antitumor efficacy. *S. typhimurium* A1-R is auxotrophic for leu-arg which prevents it from mounting a continuous infection in normal tissues. A1-R has no other attenuating mutations as does VNP20009, and therefore, has very high tumor-targeting capability. A1-R was able to eradicate primary and metastatic tumors in monotherapy in nude mouse models of prostate, breast, and pancreatic cancer, as well as sarcoma and glioma (7-13). Tumors with a high degree of vascularity were more sensitive to A1-R and vascular destruction appears to play a role in A1-R antitumor efficacy (14).

A random library of *Salmonella enterica typhimurium* 14028 genomic DNA was previously cloned upstream of a promoterless gene encoding the green fluorescent protein (GFP). A population of *Salmonella* containing this library was injected i.v. into tumor-free nude mice and into human PC-3 prostate tumors growing subcutaneously in nude mice. Fluorescence-activated cell sorting was used to enrich for bacterial clones expressing GFP from spleens or tumors. Three candidate tumor-specific promoter clones were individually tested *in vivo*, using GFP imaging. Two of the three clones (*pflE* and *ansB* promoters) were induced in hypoxic conditions found in tumors (15).

The relative fitness of 41,000 *Salmonella* transposon insertion mutants growing in mouse models of human prostate and breast cancer was also previously tested. Two classes of potentially non-toxic mutants were identified. Class 1 mutants showed reduced fitness in normal tissues and unchanged fitness in tumors. Class 2 mutants showed reduced fitness in tumors and normal tissues. A class 1 mutant (*STM3120*) effectively targeted tumors after intragastric delivery, suggesting an oral route as an option for bacterial cancer therapy (16). A similar finding of effective oral delivery of *S. typhimurium* for cancer therapy was recently made by Jia and coworkers (17).

Although Coley's toxins and bacteria themselves may act as immune stimulators, the experiments of Flentie et al (4), and other experiments described above, demonstrate that bacteria such as *S. typhimurium* directly attack and kill tumors. Tumor-targeting bacteria need to be attenuated to be non-toxic, but not over-attenuated in order not to reduce antitumor efficacy.

Further development of the technology described by Flentie et al. (4) and Arrach et al. (15) are also possible. For example, combinations of two or more promoters that are preferentially induced in tumors by different regulatory mechanisms would allow the delivery of two or more toxins, possibly sequentially. Using highly-selective tumor-targeting bacteria such as *S. typhimurium* A1-R, inducible *Salmonella* promters could be combined with tumor-specific *Salmonella* promoters for controlled expression and greater efficacy.

In addition, use of GFP for imaging the bacteria offers advantages of real-time visualization of single bacteria *in vivo* (18) which could lead to selection of enhanced cancer cell-targeting variants of *S. typhimurium*. For example, dual-color labeling of the cancer cells with GFP in the nucleus and red fluorescent protein (RFP) in the cytoplasm, allows simultaneous imaging of intracellularly-infecting GFP-expressing bacteria and apoptotic behavior of the infected cancer cells (Figure 1).

That tumor characteristics which are barriers to standard therapy, are facilitators of bacterial therapy, demonstrate that “bugging tumors” has great promise for treatment of cancer.

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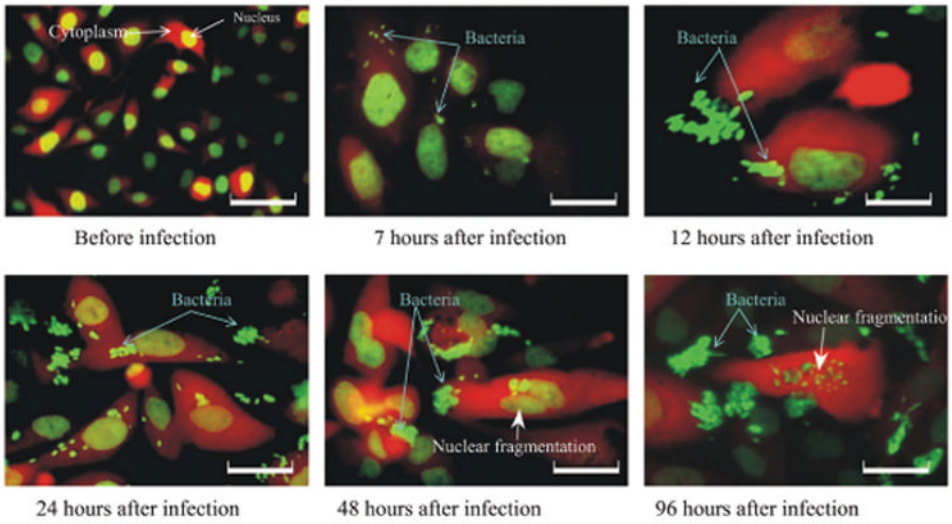

Figure 1.

Intracellular growth of *S. typhimurium* A1. Time course of GFP-labeled *S. typhimurium* A1 growing in GFP–RFP-labeled PC-3 human prostate cells *in vitro*. PC-3 human prostate tumor cells were labeled with RFP in the cytoplasm and GFP in the nucleus by means of a fusion with histone H2B. Interaction between bacteria and tumor cells was observed at the indicated time points under fluorescence microscopy magnification. (Bar: 156 μm for *Upper Left*; otherwise, Bar: 78 μm) (7).