the use of tumor-specific promoters (\textsuperscript{5}) that they had identified to show well as by the acidic pH often found in tumors. Bacteria to target cancer (\textsuperscript{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 bacterial cancer therapy, the future of bacterial therapy of cancer appears bright. Cancer Discov; 2(7); 588–90. © 2012 AACR.

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A random library of \textit{Salmonella} \textit{typhimurium} 14,028 genomic DNA was previously cloned upstream of a promoterless gene encoding GFP. A population of \textit{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. Candidate tumor-specific clones were identified and 3 were individually tested \textit{in vivo}, using GFP imaging. Two of the 3 clones (\textit{pflE} and \textit{ansB} promoters) were induced in hypoxic conditions found in tumors (15).

The relative fitness of 41,000 \textit{Salmonella} transposon insertion mutants growing in mouse models of human prostate and breast cancer was also previously tested. Two classes of potentially nontoxic 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 (\textit{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 \textit{S. typhimurium} for cancer therapy was recently observed by Jia and colleagues (17).

Although Coley’s toxins and bacteria themselves may act as immune stimulators, the experiments of Flentie and colleagues (4) and other experiments described above show that bacteria such as \textit{S. typhimurium} directly attack and kill tumors. Tumor-targeting bacteria need to be attenuated to be nontoxic but not overattenuated in order not to reduce antitumor efficacy.

Further development of the technology described by Flentie and colleagues (4) and Arrach and colleagues (15) is also possible. For example, combinations of 2 or more promoters that are preferentially induced in tumors by different regulatory mechanisms would allow the delivery of 2 or more toxins, possibly sequentially. Using highly selective tumor-targeting bacteria such as \textit{S. typhimurium} A1-R, inducible \textit{Salmonella} promoters could be combined with tumor-specific \textit{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 \textit{in vivo} (18) that could lead to selection of enhanced cancer cell-targeting variants of \textit{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 (Fig. 1).

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

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**Figure 1.** Intracellular growth of \textit{S. typhimurium} A1. Time course of GFP-labeled \textit{S. typhimurium} A1 growing in GFP-RFP-labeled PC-3 human prostate cells \textit{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. Scale bar, 156 μm for top left; otherwise, scale bar, 78 μm (7).
REFERENCES


