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Bacteria and genetically modified bacteria as cancer therapeutics: Current advances and challenges

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ABSTRACT

Bacteria act as pro- or anti- tumorigenic agents. Whole bacteria or cytotoxic or immunogenic peptides carried by them exert potent anti-tumor effects in the experimental models of cancer. The use of attenuated microorganism(s) *e.g.*, BCG to treat human urinary bladder cancer was found to be superior compared to standard chemotherapy. Although the phase-I clinical trials with *Salmonella enterica* serovar *Typhimurium*, has shown limited benefits in human subjects, a recent pre-clinical trial in pet dogs with tumors reported some subjects benefited from this treatment strain. In addition to the attenuated host strains derived by conventional mutagenesis, recombinant DNA technology has been applied to a few microorganisms that have been evaluated in the context of tumor colonization and eradication using mouse models. There is an enormous surge in publications describing bacterial anti-cancer therapies in the past 15 years. Vectors for delivering shRNAs that target oncogenic products, express tumor suppressor genes and immunogenic proteins have been developed. These approaches have showed promising anti-tumor activity in mouse models against various tumors. These can be potential therapeutics for humans in the future. In this review, some conceptual and practical issues on how to improve these agents for human applications are discussed.

1. Introduction

Chemotherapy, although widely used for treating many tumors, causes many off-target effects such as significant damage to normal tissues. In contrast biological therapeutics, such as antibodies, peptide-mimetics and a few cytokines, generally exert target-specific effects and are relatively safer for human use. Although attenuated viruses and bacteria have been successfully used for generating immunity to potential pathogens, their general use for tumor therapy is still lagging. The isolation of *Streptococcus pyogenes* from sarcoma by Busch [1] and neck cancer by Coley [2] are historical landmarks that suggested the potential use of bacteria to treat human cancers. The use of microorganism(s) to treat human cancers provided a short term benefit, but eventually tumors recurred [3]. Recombinant viruses with tumoricidal activity have been developed over the years. Major obstacles with these viral vectors include lack of the specificity toward cancer cells, short half-life of the vector, limited tropism for antigen-presenting cells, pre-existing immunity, rapid development of neutralizing antibodies to the viral coat proteins, etc., which limits their utility as vehicles to elicit tumoricidal activities and/or gene therapy.

A major step in the development of bacterial therapeutics is the identification of potential species and strains with minimal pathogenicity to the host. In the past century, many genera of bacteria have been isolated and/or identified in and around various tumors (see Cummins and Tangney [4] for additional details).

Abbreviations: BC, breast cancer; CC, cervical cancer; CMV, cytomegalovirus; CRC, colorectal cancer; HCC, hepatocellular cancer; Hly, hemolysin, an exotoxin produced by bacteria.; HSC, hematopoietic stem cells; LC, lung cancer; LPS, lipopolysaccharide; PC, prostate cancer; PSA, prostate-specific antigen; RCC, renal cell cancer; SCC, squamous cell cancer; SPI, *Salmonella* pathogenicity islands; DLT, dose-limiting toxicity – a single minimal dose of a therapeutic agent that results in unacceptable side effects or toxicity in a cohort; MTD, maximum tolerated dose – a single maximal dose of a therapeutic agent that produces the desired response(s) with acceptable side effects or toxicity in a general population. This value is less than DLT; Exotoxins, a family of bacterial proteins secreted or injected using type-I/II secretory apparatus targeting specific host components. Toxins that target host cytosolic proteins include Cholera, Diphtheria, Pertussis and Shiga toxins while another group that creates pores on host cell membrane includes cholesterol-dependent cytolysins and RTX toxins; Epitope spread, a phenomenon where multiple antigen-specific immune responses are triggered that are unrelated, in sequence or structure, to the primary immunogen.

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Extracellular and intracellular bacteria, both gram-positive and gram-negative, have been isolated. The ability of such bacteria to successfully colonize regions of implanted tumors in experimental animals has also been addressed and only a few had the potential to home into tumors [5] (see Table 1). Upon bacterial administration, each of these preferentially accumulated and grew in tumor vicinity in a time-dependent manner when compared to most other host tissues (Tables 2 and 3). Hence, it is possible that the tumor microenvironment may be more conducive for bacterial survival and/or growth as it may provide protection from the host immune system and/or nutrients (see Table 1). Even though the precise mechanism behind this selective homing is not understood, hemodynamics [6] and/or chemotaxis [7] appear to be essential for this process. Survival and growth of bacteria homed into tumors appears to be dependent on their oxygen requirement and other characteristics such as intra/extracellular mode of survival, and motility. So far, facultative and obligate anaerobic, intracellular and extracellular bacteria have shown tremendous success in colonizing tumors implanted in mouse. In this review we will focus more on *Salmonella enterica* subsp *enterica* serovar *Typhimurium* (hereafter *S. Typhimurium*), a rod-shaped flagellated gram-negative non-spore forming facultative anaerobic bacteria, closely related to *Escherichia coli* a commensal in the intestines of vertebrates, as a broad spectrum vaccine host and in cancer therapy/immunity.

The genus *Salmonella*, a gammaproteobacterial member, is represented by two species *viz*., *bongori* and *enterica*. There are six subspecies *viz*., *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*, of which subspecies *enterica* is a medical concern. The subspecies *enterica* is further classified into typhoidal serovars (represented by *Typhi*, *Paratyphi* (A, B, C), *Sendai*, *etc*.) and non-typhoidal serovars (NTS, represented by *Choleraesuis*, *Dublin*, *Enteritidis*, *Infantis*, *Typhimurium*, *etc*.) based on biochemical signatures on flagella, capsule, carbohydrates and LPS. More than 2500 serovars of *S. enterica* have been described. In a natural setting, the typhoidal serovars cause enteric or typhoid fever, a systemic disease mostly restricted to humans, while the NTS that cause a self-limiting enteritis and/or diarrhea is prevalent in many mammals. Currently, two commercially available vaccines (one administered orally and the other injectable) against serovar *Typhi* are used more commonly in poultry and swine industries. Although both of these vaccine formats are safe for human use, it requires regular boosters to maintain effective immunity. Thus, the collective natural features and the ease of genetic manipulation of *S. Typhimurium* have been exploited for targeting experimental tumors in mouse models. Such modified strain(s) *i.e*., rendered less toxic, when administered intravenously has the potential to target distant tumors.

Table 1
Bacteria home and replicate in tumor microenvironment.

Bifidobacteria	
B. adolescentis	
B. animalis	
B. bifidum	
B. boum	
B. breve	
B. coryneforme	
B. dentium	
B. indicum	
B. infantis	
B. longum	
B. magnum	
B. pseudolongum	
Lactobacilli	
L. bifidus	
L. delbrueckii	
Clostridia	
C. absonum	
C. acetobutylicum	
C. bifermentans	
C. difficile	
C. histolyticum	
C. perfringens	
C. novyi – exhibited extensive spreading even in poorly-vascularized tumor areas	
C. sordellii – exhibited extensive spreading even in poorly-vascularized tumor areas	
Murine tumors	Tumor:Liver
B16 melanoma	12,000:1
M27 lung carcinoma	10,000:1
Spontaneous breast tumor	700:1
Human tumor xenografts	
MDA-MB-231 breast carcinoma	34,000:1
DU145 prostate	24,000:1
HCT 116 colon carcinoma	17,000:1
DLD1 colon carcinoma	15,000:1
HTB177 lung carcinoma	4000:1
LOX melanoma	3000:1
A549 lung carcinoma	300:1
SW-620 colon carcinoma	275:1

Based on Dang et al. [5] and Sznol et al. [21] with minor modifications.

As the major cell wall component of gram-negative bacteria, LPS is a key determinant to the pathogen's success in colonizing host tissues *vis-à-vis* infection. Biochemically LPS is a phosphoglycolipid; it can be separated into three modules *viz*., a central core oligosaccharide, a peripheral variable polysaccharide and a lipid portion. The central portion of core oligosaccharide is composed of 8-carbon sugars called KDO (3-deoxy-D-mannooctulosonic acid). One of the 8-carbon sugars is covalently linked to a 6-carbon sugar while the opposing 8-carbon sugar is covalently linked to a 7-carbon sugar. Core oligosaccharide is highly diverse among bacterial species or even within strains of a bacterial species [8]. The 6-carbon sugars are acylated to form the Lipid A component that is embedded in the bacterial outer membrane. The other side containing the 7-carbon sugars is glycosylated to form the O antigen that projects into the extracellular medium (Fig. 1). The O antigen is a highly variable polysaccharide component that forms the basis of 'Rough' and 'Smooth' strain classification. The core oligosaccharide and 6-carbon sugars attached to the Lipid A bear phosphate residues that are essential for virulence. The common feature among the experimental strains is that they are rendered less toxic *i.e*., well tolerated, to host cells as they are administered in high doses directly into systemic circulation. This has been accomplished either by direct inactivation of genes coding for the enzymatic components responsible for the linkages in the LPS or by indirect means by targeting gene products that contribute to survival in specific niches (Table 2 and Fig. 1). Since these two components are interdependent virulence factors, strong immune reactions from the host are dampened. In these strategies, bacteria are selected for those that retain the ability to colonize host tissues *i.e*., tumors and incapable of causing disease *e.g*., *S. Typhimurium* strain VNP20009 cannot synthesize wild-type LPS, so it is less immunogenic to humans while strain LH430 is deficient in *pho* regulators; a two-component system essential for environmental sensing, adaptation and survival [9]. Also some strains are engineered with an additional mutation(s) in other locus/loci to prevent unanticipated reversion to wild-type genetic background (Table 2) *e.g*. strain VNP20009 is also a purine auxotroph. However, reversion is common in strains mutant for aromatic and/or hydrophobic amino acid metabolism; though the mechanism remains unknown. Hence, such vectors are not suitable for targeting tumors growing *in vivo*.

The development of an attenuated serovar *Typhimurium* VNP20009 that retained the ability colonize experimental tumors in mice without eliciting strong immune and/or toxic side effects paved way for phase-I clinical trials in humans [10–12] and

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