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Review

Bacterial-directed enzyme prodrug therapy

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ABSTRACT

Current conventional treatments for cancer lack tumour selectivity resulting in the destruction of healthy tissue and severe adverse effects to the patient in addition to limiting the administration dose and efficacy. Hence, it is imperative that we seek alternative approaches to treat cancer that localise therapeutic agents to the site of the tumour and spare normal tissue. The use of bacteria in cancer therapy represents one such approach. Bacteria were first used as anti-cancer agents over a century ago. Today, this field has re-emerged from the past and is progressing at a rapid rate. Bacteria are used as anticancer agents either alone or in combination with conventional treatments and have been armed with an arsenal of therapeutic genes, which enhance their efficacy. Bacterial directed enzyme prodrug therapy (BDEPT) is one of the most promising approaches, which harnesses the tumour-specific location of bacteria to locally activate systemically administered 'prodrugs' within the tumour in order to induce selective tumour destruction. BDEPT is a relatively new concept. It was originally conceived more than 10 years ago but it is only until recently that we witness a surge in activity in this field. In this review, we provide a full account of developments in the field of BDEPT since its inception. We share technical knowhow and discuss optimization strategies for vector and enzyme combinations, provide a clear view of the research landscape and suggest possible directions for the field.

Contents

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1. Introduction

The discovery that bacteria can infect and attack tumours dates back 150 years. In both Europe and America, it was initially observed that some cancers regressed following accidental *Streptococcus pyrogenes* infections that occurred in hospitalised patients. William Coley was an American physician that pioneered the work and devoted his entire career in investigating the use of bacteria as an alternative method of cancer treatment [1,2]. Despite his success, he was never able to develop a perfect system and therefore, the general interest in bacteria as anticancer agents eventually faded. Nonetheless, Coley's findings provided the foundation for two disparate modern fields; i) immunotherapy and ii) bacterial cancer therapy.

1.1. Nature of tumour-specific bacterial growth

The selectivity of bacterial growth within tumours relates to a tissue phenotype that distinguishes tumour tissue from healthy tissue. Ironically, the microenvironment of the tumour which protects it from most anticancer treatments represents the 'Achilles heel' that sensitises it to bacterial anticancer agents. It is well documented that different bacteria preferentially accumulate in various experimental tumours. For example *Salmonella* strain VNP20009 has demonstrated ratios of tumour to normal tissue of 300–25,000:1 [2–4]. Various theories have been proposed in order to explain such observations [5]. The primary factors that underpin this specificity are direct or indirect results of tumour growth processes ultimately resulting in zones of necrosis. In order for tumours to grow and develop, they require new blood vessels to be formed, a process known as neoangiogenesis. It is a hallmark of cancer and essential for the continued supply of oxygen and nutrients to the tumour [6]. Once the tumour radius reaches a critical mass, oxygen can no longer adequately reach the inner layers of the tumour, and the cells become gradually hypoxic. In the hypoxic zone, the low-oxygen partial pressure induces further angiogenesis. These newly formed vessels are abnormal in structure and function [6] and create physiological barriers to the delivery of therapeutic agents, and immune cells [7]. One of the exploitable features of their abnormality is that they consist of pores of various sizes ranging from 200 nm to 2 μm (depending on the tumour) [8]. This potentially allows micro-organisms such as bacteria to egress from the vasculature and lodge locally within the tumour mass. Necrotic regions are areas of dead cells usually but not exclusively found in the middle of the tumour mass. Such zones are permissive for bacterial growth as they would be expected to provide protection from the immune system and sufficient nutrients (e.g. purines) from the dead tumour cells. Indeed, surgeons have reported anecdotally, some tumours (usually large with extensive necrotic regions) producing a decaying odour upon surgical resection, most likely originating from infecting microorganisms.

The exact location of bacterial proliferation within the tumour may vary between species. A recent 3D imaging study indicated the growth of anaerobic bifidobacteria as multiple clusters within non-viable tumour regions [9]. Evidence by Forbes et al. [10] demonstrated that salmonellae proliferated within the necrotic areas of model tumours. Such an observation implies that their use is limited to large tumours. However, this contradicts earlier data published by [3] and recent data by [11], which demonstrate *Salmonella* proliferation in both normoxic and hypoxic areas. Such a capacity is preferred in a clinical context. An ideal bacterial anticancer agent should target to and proliferate within micrometastatic tumours which naturally lack necrotic regions. For example, *Escherichia coli* K12 MG1655 and HJ1020 tagged with light emitting genes have been shown to target very small tumours as well as large ones [12] and even anaerobic *Bifidobacterium breve* has displayed a similar capacity [13].

It appears that many different types of bacteria can proliferate specifically within tumours e.g. *Magnetospirillum magneticum* [14], *E. coli* CFT073, *E. coli* Top10 and *Salmonella flexneri* 2a SC602 [15]. *Vibrio cholerae*, *Listeria monocytogenes*, *Salmonella enterica SL7202*, and even, *E. coli* DH5α have all been shown to replicate within mouse xenograft tumours [16], suggesting that there is a possibility for most types of bacteria administered to tumour-bearing mice to find a safe haven and proliferate within the tumour, therefore giving the impression of "targeting" [9,17–19].

1.2. Why bacterial-mediated therapy?

The use of bacteria in cancer therapy may be favourable over other microorganisms such as gene therapy vectors derived from viruses or over standard chemotherapy for a number of reasons. Firstly, several bacterial species are motile and have the ability to swim against pressure or diffusion gradients created within the abnormal tumour environment. Small drug molecules or viruses, on the other hand, rely on convection in order to spread within the tumour. Hence, the interstitial pressures which exist in tumours limit their penetration significantly [20]. Secondly, bacteria can adhere to or invade tumour cells and are also capable of proliferating within the tumour area establishing extracellular colonies. Furthermore, their large genome allows them to accommodate a variety of exogenous therapeutic genes (for example, prodrug activating enzymes and cytokines). Most importantly from a clinical safety point of view, they can be killed with antibiotics (e.g. Metranidazole) if complications arise following treatment. By contrast, viral vector capacity can be limited and if adverse side effects do arise, viruses cannot be eliminated by antibiotics.

2. Exploitation of tumour-targeting bacteria

2.1. Innate oncolytic activity

Long after Coley's work, the interest in the use of bacteria to treat cancer was re-ignited as it was observed that the tumour microenvironment favoured bacterial growth. In experimentally-induced tumours in mice, it was shown that bacteria were able to proliferate in specific areas within the tumour inducing lysis of the surrounding tissue.

2.1.1. Clostridia

A number of studies in the mid-20th century have shown that Gram-positive obligate anaerobic clostridia could proliferate in the hypoxic or necrotic areas of tumours and thus, were investigated as oncolytic agents for the treatment of cancer. Clostridia are spore-forming anaerobic bacteria that need to be injected to the patient as spores. These spores then travel to the tumour site and only germinate if there is an anoxic region present (note: such regions are only present in large tumours). One of the first strains to be tested as an anticancer agent was *Clostridium histolyticum*. Direct injection of spores in mice sarcomas induced noticeable tumour regression and lysis [21]. However, the actual microscopic observation that bacteria proliferated within the tumour was made a few years later using the extremely virulent strain of *Clostridium tetani*. Despite its ability to shrink tumours, this species elicited a high toxicity following injection and resulted in rapid death of the tumour-bearing mice [22]. Researchers then opted to change to the non-pathogenic strain *Clostridium butyricum*, 'M55' [23] whose non-pathogenic nature