ELSEVIER

Institut Pasteur

Research in Microbiology xx (2015) 1–11

www.elsevier.com/locate/resmic

Review

The potential of clostridial spores as therapeutic delivery vehicles in tumour therapy

Aleksandra M. Kubiak, Nigel P. Minton*

Clostridia Research Group, BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), School of Life Sciences, The University of Nottingham, Nottingham NG7 2RD, UK

Received 4 September 2014; accepted 15 December 2014

Abstract

Despite substantial investment in prevention, treatment and aftercare, cancer remains a leading cause of death worldwide. More effective and accessible therapies are required. A potential solution is the use of endospore forming *Clostridium* species, either on their own, or as a tumour delivery vehicle for anti-cancer drugs. This is because intravenously injected spores of these obligate anaerobes can exclusively germinate in the hypoxic/necrotic regions present in solid tumours and nowhere else in the body. Research aimed at exploiting this unique phenomenon in anti-tumour strategies has been ongoing since the early part of the 20th century. Only in the last decade, however, has there been significant progress in the development and refinement of strategies based on spore-mediated tumour colonisation using a range of clostridial species. Much of this progress has been due to advances in genomics and our ability to modify strains using more sophisticated gene tools.

Keywords: Cancer therapy; Spores; Clostridia; Hypoxia; Prodrug; Prodrug converting enzyme

1. Introduction

Cancer is a broad term for a class of diseases characterised by uncontrolled cell division, leading to abnormal growth. In a normal cell there is a finely regulated balance between all signals promoting or restraining growth. In tumour cells, however, this balance is disrupted. The great proportion of all abnormalities which occur in organisms are triggered either by inherited genetic predispositions, environmental factors or exposure to certain chemicals or harmful conditions which alter the genomic structure and processes [1,2]. According to the World Health Organisation it was reported that for 2012 there were 14 million new cancer cases occurring worldwide, which contributed to around 8.2 million deaths. It is predicted that within the next 15 years the burden of cancer will increase to 23.6 million.

To date, there is no single treatment for cancer. There are different ways to treat cancer, including surgery, radiotherapy, chemotherapy, hormone therapy, and immunotherapy. These approaches are effective in the management of many cases, but it can be difficult to control tumours in some patients, and produce a favourable outcome.

Cancer treatments currently in use suffer from number of limitations. Chemotherapy regimes typically result in unpleasant physical side effects (nausea, impotence), increased risk of developing other types of cancer and mild cognitive impairments [3]. Thus, medical and scientific research investigating new anti-cancer strategies continues.

A particularly innovative approach that is less collateral to patient' general health and well-being is gene therapy. Although, still nascent, this new group of treatments might bring more targeted (specific) approach to treat a specific cancer type and provide better cure than the standard methods.

The concept of using proteins as anti-cancer agents has led to a multitude of strategies reliant on the use of toxins,

* Corresponding author.
E-mail address: nigel.minton@nottingham.ac.uk (N.P. Minton).

http://dx.doi.org/10.1016/j.resmic.2014.12.006
0923-2508/

cytokines, immunogens or enzymes. Whilst such agents are invariably highly effective against tumour cell lines, the more exigent problem remains their selective delivery into the tumour mass. Accordingly, much of the focus of gene therapy research has been devoted to the derivation and testing of tumour delivery vehicles, most notably antibody-based, viral, non-viral or non-biological delivery systems. Their characteristics and specificities, as well as advantages and drawbacks, have been the subject of a number of reviews [4–6]. Nevertheless, it is worth noting that these delivery strategies have created concerns over universality, safety and development costs. Additionally, the low success rate of these delivery vehicles to selectively target the microenvironment of a tumour is a key reason that many studies have been terminated after stages I or II of clinical trials.

2. Targeting tumour hypoxia

It's estimated that more than 80% of cancers are classified as solid tumours [7]. The majority of these contain large regions of poorly oxygenated tissue, known as hypoxia. These hypoxic tumour tissues occur due to a persistent imbalance between the oxygen being delivered to the tumour through blood microvessels and that being consumed by proliferating cancer cells. In addition, some solid tumours are found to have necrotic (dead) cores, a direct effect of long-standing tissue hypoxia [8,9].

Tumour hypoxia has been demonstrated to reduce the efficacy of many standard cytotoxic drugs used in the treatment of cancer [10]. This is due to ineffective penetration of the drug into the hypoxic mass of the tumour, due to poor vascularisation. A chosen drug concentration may be inadequate for the destruction of the more distant, more hypoxic cells in the interior of the tumour.

Growing evidence suggests hypoxia acts as a prognostic for patient survival and cancer control [11]. The study of Nodsmark et al. produced evidence supporting the idea that tumour hypoxia was linked with a negative prognosis in 397 patients examined with advanced head and neck cancer following primary radiotherapy [12]. Similar findings were found for soft tissue sarcomas and cervix cancers [13,14].

Cancer survival prognosis is strongly related to the development of metastatic disease. It is estimated that around 90% of cancer-related deaths are linked to metastatic spread [15,16]. Hypoxia plays a key role in metastatic progression [11,17]. This is mainly due to chaotic angiogenesis of new blood vessels that alters the tissue microenvironment, the hypoxia-stimulated expression of pro-metastatic genes or hypoxia-triggered mutagenesis of p53 gene [11].

Patients diagnosed and surgically treated for primary tumours with severe hypoxia have a much lower chance of disease-free survival. There is a strong likelihood that metastasis has been initiated, which goes undetected at the time of surgery [13].

The challenge of hypoxia has provided opportunities for novel tumour therapies [11]. The presence of tissue with significantly reduced levels of oxygen and necrosis are unique to solid tumours. This can be exploited as a niche environment for cancer therapies. Certain novel strategies involve the use of hypoxia-activated prodrugs or development of HIF-1 inhibitors. A particularly promising option is the use of anaerobic bacteria. An oxygen-free environment creates ideal conditions for certain microbes, such as *Clostridium* spp., to colonise and target a necrotic/hypoxic growth. Such a therapy could potentially be used to treat relatively small tumours, preventing further development of hypoxic cancer tissue and the subsequent metastatic progression.

3. A brief historical overview on clostridial oncolysis

3.1. The use of clostridia species as a tool in cancer therapy

The genus *Clostridium* is one of the largest prokaryotic genera, consisting of more than 130 physiologically diverse Gram-positive bacterial species. They are obligate, rod-shaped anaerobes and, in common with the aerobic genus *Bacillus*, produce endospores in order to survive adversity [18].

As a grouping, *Clostridium* is, in the main, composed of entirely benign, non-pathogenic species that are widely distributed in the environment. Nonetheless, they are best known for the pathogenic properties of a handful of species. It is the attributes of the non-pathogenic members, however, which may ultimately be of most significance to mankind [19]. In particular, it is the ability of clostridial endospores to infiltrate, and hence selectively germinate in, the hypoxic regions of solid tumours that may have the greatest impact [20,21]. The history of the application of clostridial spores in cancer therapies has been broadly reviewed in a number of scientific publications [22–26]. Some of the most crucial findings, which laid the foundation for the most recent advances in clostridial tumour therapy, are briefly outlined in Box 1.

To avoid the use of pathogenic species, such as *Clostridium histolyticum* and *Clostridium tetani*, the use of *Clostridium butyricum* M-55 was investigated by Möse and Möse in 1959. Significant oncolytic effects were observed after injecting spores into animals and later into human patients [27–29]. Thanks to these properties, the strain was named *Clostridium oncolyticum* and later reclassified as *Clostridium sporogenes* M-55 (ATCC 13732). Saccharolytic clostridia such as *Clostridium beijerinckii* or *Clostridium acetobutylicum* were also investigated and proved to be effective in tumour colonisation. However, compared to *C. sporogenes* the reproducibility of colonisation was inferior. Moreover, the total number of vegetative cells present in a tumour was orders of magnitude lower than *C. sporogenes* [30]. The initial benefits of a more aggressive coloniser have additionally been shown in a study that utilised a non-toxinogenic strain of *Clostridium novyi-NT*. The superior colonisation abilities of *C. sporogenes* and *C. novyi-NT* are likely a consequence of them being proteolytic species.

Please cite this article in press as: Kubiak AM, Minton NP, The potential of clostridial spores as therapeutic delivery vehicles in tumour therapy, Research in Microbiology (2015), http://dx.doi.org/10.1016/j.resmic.2014.12.006