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Review

Facultative or obligate anaerobic bacteria have the potential for multimodality therapy of solid tumours

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ABSTRACT

Recent understanding of the unique pathology of solid tumours has shed light on the difficult and disappointing nature of their clinical treatment. All solid tumours undergo angiogenesis that results in biological changes and adaptive metabolisms, *i.e.* formation of defective vessels, appearance of hypoxic areas, and emergence of an heterogeneous tumour cell population.

This micro-milieu provides a haven for anaerobic bacteria. The strictly anaerobic clostridia have several advantages over other facultative anaerobes such as salmonella or lactic acid-producing, Gram-positive, obligate, anaerobic bifidobacteria. Both pathogenic and non-pathogenic clostridia have been demonstrated to specifically colonise and destroy solid tumours. Early trials of non-pathogenic strains in humans had shown plausible safety. Genetic modifications and adaptation of pathogenic and non-pathogenic strains have further created improved features. However, these manipulations rarely generate strains that resulted in complete tumour control alone. Combined modalities of therapies with chemo and radiation therapies, on the other hand, often perform better, including 'cure' of solid tumours in a high percentage of animals.

Considering that clostridia have unlimited capacities for genetic improvement, we predict that designer clostridia forecast a promising future for the development of potent strains for tumour destruction, incorporating mechanisms such as immunotherapy to overcome immune suppression and to elicit strong anti-tumour responses.

1. Introduction

The word *tumour* originated from Latin *tumor*, meaning 'swelling'. Solid *tumours* are masses of 'swellings' made up of abnormal cells characterised by unrestricted growth in at least three different tissue compartments – the original compartment (primary tumour); the mesenchyme at the primary site (tumour invasion); and distant epiderm, endoderm and mesenchyme (tumour metastasis).[1]

Ninety percent of all human cancers are solid tumours. The initial avascular mass is harmless, but when it grows to and exceeds about 2 mm in diameter, the local vasculatures

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of the surrounding normal tissues become inadequate to support the growing tumour mass.[2] Consequently, some tumour cells are deficient in nutrients and oxygen as well as in accumulating acidic waste products, triggering cellular release of tumour angiogenic factors (TAFs). The diffusion of these TAFs into the surrounding normal tissues stimulates the endothelial cells of the nearby blood vessels to differentiate, divide, and migrate from their original basal lamina, enter the extracellular matrix, and eventually migrate towards the tumour site. Strands of such cells are formed and inter- and intracellular lumina developed to give rise to capillary tubules, which together form a network of new vessels.[2,3] The tumour mass thus becomes vascularised and new blood flow again established although the normal efficient vascular architecture expected is disturbed and is chaotic inside the growing tumour mass leading to areas of tumour hypoxia, acidity, nutrient deficiency and cell death.

2. Angiogenesis and hypoxia – the twin devils of the unique solid tumour pathology

Angiogenesis is thus fundamental for the continuation of local growth, and eventual metastatic spread of solid tumours.[3] These events are coordinated with several TAFs involved. Amongst them, vascular endothelial growth factor (VEGF) is a key stimulator, which exhibits its effect on the vasculature in paracrine and autocrine fashions.[3,4] It not only induces the sprouting, proliferation and outgrowth of capillary endothelial cells, but also increases the permeability of the capillaries and antagonises apoptosis of endothelial cells.[4,5]

In addition to angiogenic events, tumours establish their blood supply via a number of other processes as well. These include vasculogenesis, vascular remodelling, intussusception and possibly vascular mimicry by tumour cells in certain tumours.[6] As a result, the blood vessels become abnormal in structure and function while some are known to have enlarged pore sises, leaky micro-vessels and incomplete ends and yet others become a jumbled mass – thick in some areas and pinched at others.[7] As the tumour mass continues to grow, the diffusion distances between the nutritive microvessels and the number of tumour cells increase and the oxygen transport capacity of the blood is thereby reduced, due to the presence of disease or treatment related anaemia. As a result, this chaotic pathophysiology creates an unbalanced, erratic or hindered blood supply and significantly inefficient perfusion, causing many regions within the tumours to become transiently or chronically oxygen deficient, a phenomenon called hypoxia.[8]

Hypoxia is now a well characterised feature and believed to exist in almost every solid tumour.[8] Most of the hypoxic areas have oxygen concentrations of 10 mmHg or less, whereas well oxygenated tissues have oxygen concentrations of 50–60 mmHg.[9,10] To cope with the low oxygen stress, tumour cells respond by converting to anaerobic metabolism, or glycolysis, which in turn produces lactic acids, resulting in lower tissue pH.[11] Prolonged hypoxia can also increase genomic instability and genomic heterogeneity. These adaptive genomic changes allow some tumour cells to overcome nutritive deprivation or to escape from their hostile environment. These survival advantages of tumour cells can be further enhanced by genomic changes leading to loss of apoptotic potential, which may act as a selective pressure for tumour cell variants. These new variants have advantages over less adapted cells in an hypoxic micro-environment and further expand through clonal selection, often becoming the dominant cell types. These variants further intensify hypoxia, establishing a vicious cycle of hypoxia, malignant progression, and treatment resistance.[10]

Hypoxia can also act in an epigenetic fashion, further altering gene expression, such as stimulation of VEGF expression, in which the transcription factor hypoxia-inducible factor 1 (HIF-1) plays a major role. Under hypoxic conditions, HIF-1 upregulates the transcription of VEGF and stabilises its mRNA.[12] Furthermore, hypoxia-induced pathways influence VEGF activity by post-transcriptional regulation of VEGFR-2 and by hypoxia-inducible expression of VEGFR-1.[12] These changes in VEGF upregulation are believed to be associated with increased aggressiveness and metastasis of the solid tumours.

Hypoxia represents one of the most pervasive stresses, eventually resulting in tissue remodelling. Consequently, such a situation arises at the tumour site, *e.g.* the inner mass of the tumour is actually necrotic and this is surrounded by a quiescent cell region while the outer layer is made up of the actively proliferating tumour cells.[7,13,14] All of these abnormalities, *i.e.* the structuring of the primary tumour and the metastatic variants, play a fundamental role in treatment failure with current approaches.[10]

3. Facultative anaerobic and obligate anaerobic bacteria have unique abilities for tumour targeting and tumour lysing

The unique micro-milieu of solid tumours can in fact be turned to advantage as it was noticed that hypoxic regions of tumours fall into three major groups (Table 1):

3.1. *The lactic acid-producing, Gram-positive obligate anaerobic bacteria represented by bifidobacteria*

Three species of the common flora of the human intestine were the foci of past studies, *B. longum*, *B. infantis* and *B. adolescentis*.[15,16] Initial 'proof of concept' testing for the use of bifidobacteria in cancer therapy was by intravenous injection of 5×10^6 colony forming units (CFU) of *B. longum* into mice implanted with Ehrlich ascites tumours. The bacteria were shown to be highly selective and localised primarily within the tumour cells, with virtually no bacteria in other organs after 96 h. At 1 h, 10^2 CFU/g tumour tissues were present, which increased to 10^6 CFU/g by day 7. Unfortunately, no obvious oncolytic effect was observed.[15,16] Later on, *B. adolescentis* was shown to markedly induce tumour apoptosis and prevent occurrence and development of colorectal carcinoma *in vivo* in animal models.[17] The shortcoming of using bifidobacteria for cancer therapy, though, is that they are non-spore formers, and thus are more susceptible to non-permissive conditions and more difficult to store and handle.