



Bacteriotherapy of cancer

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ABSTRACT

Resistance to conventional anticancer therapies in patients with advanced solid tumors has prompted the need of alternative cancer therapies. Moreover, the success of novel cancer therapies depends on their selectivity for cancer cells with limited toxicity to normal tissues. Bacteriotherapy is the treatment of disease by the use of bacteria or their products. It may have potential applications for cancer therapy as it is the most potential and promising strategy is bacteria based gene-directed enzyme prodrug therapy. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Bacteriotherapy;
Cancer.

INTRODUCTION

Conventional anticancer therapies such as chemotherapy are losing their sheen in the battle against cancer. Therefore, strategies for treatment of cancer need to be constantly modified to fulfill the growing demands of alternative therapies^[17].

Specific delivery of therapeutic enzymes to cancer cells for subsequent activation of anticancer drugs in the tumor microenvironment is a promising approach to improve the selectivity of cancer chemotherapy. Different vehicles including viruses, liposomes and antibodies have been evaluated to deliver therapeutic enzymes to cancer cells^[6].

Historically, bacteria were used as oncolytic agents for malignant brain tumours. Advances in bacteriology and molecular biology have widened the scope of bacterial approaches to cancer therapy and various possibilities include the use of bacteria as sensitising agents for chemotherapy, as delivery

agents for anticancer drugs, and as vectors for gene therapy. Bacterial toxins can be used for tumour destruction and cancer vaccines can be based on immunotoxins of bacterial origin. The most promising approaches are the use of genetically modified bacteria for selective destruction of tumours, and bacterial gene-directed enzyme prodrug therapy. Knowledge gained from study of bacterial genomes forms an important basis of use of bacteria as anticancer agents^[10].

For at least two centuries, there have been reports that cancer patients infected with various bacteria had what appeared to be spontaneous remission. In the late nineteenth and early twentieth centuries, W.B. Coley, of what is now the Memorial Sloan-Kettering Cancer Center, pioneered bacterial therapy of cancer in the clinic with considerable success. After Coley died in 1936, bacterial therapy of cancer started to go out of favor. In the current twenty-first century, there is great resurgent interest in de-

veloping bacterial therapy for treating cancer using either obligate or facultative anaerobic bacteria. There is also controversy about which bacteria are optimum for cancer treatment and whether bacteria should be used as tumor-targeting vectors, immune stimulators, or for direct tumor killing^[7].

Recently, bacteria directed enzyme prodrug therapy (BDEPT) has been investigated for cancer therapy. Bacteria are well-suited to serve as anti-cancer agents due to their intrinsic preferential accumulation within the anoxic tumour environment, mobility, cell toxicity and immunogenicity. Furthermore, advances in biotechnology and molecular techniques have made it easier than ever to engineer bacteria as both therapeutic agents themselves and as therapeutic vectors^[21].

Clostridium

Spores of some species of the strictly anaerobic bacteria *Clostridium* naturally target and partially lyse the hypoxic cores of tumors, which tend to be refractory to conventional therapies. The anti-tumor effect can be augmented by engineering strains to convert a non-toxic prodrug into a cytotoxic drug specifically at the tumor site by expressing a prodrug-converting enzyme (PCE)^[8].

The efficacy of *Clostridium*-Directed Enzyme Prodrug Therapy (CDEPT) using nitroreductase (NTR) was demonstrated in a mouse xenograft model of human colon carcinoma. Substantial tumor suppression was achieved, and several animals were cured. These encouraging data suggest that the novel enzyme and strain engineering approach represent a promising platform for the clinical development of CDEPT.

Salmonella typhimurium

Using bacteria as therapeutic agents against solid tumors is emerging as an area of great potential in the treatment of cancer. Obligate and facultative anaerobic bacteria have been shown to infiltrate the hypoxic regions of solid tumors, thereby reducing their growth rate or causing regression. However, a major challenge for bacterial therapy of cancer with facultative anaerobes is avoiding damage to normal tissues. Consequently the virulence of bacteria must be adequately attenuated for therapeutic use^[22].

Salmonella typhimurium A1-R is auxotrophic for arg and leu, which attenuates growth in normal tissue but allows high tumor targeting and virulence. A1-R is effective against metastatic human prostate, breast, and pancreatic cancer as well as osteosarcoma, fibrosarcoma, and glioma in clinically-relevant mouse models.

Cancer treatment with attenuated *Salmonella enterica* Typhimurium (*S. Typhimurium*) has gained momentum in recent years. *S. Typhimurium* attenuated by deletion of cyclic adenosine monophosphate signaling, SalpNG.1. *S. Typhimurium* treatment reduces tumor burden and increases survival in an autochthonous breast cancer model^[5].

An alternative approach taken here is to use recombineering to make *Salmonella* not viable in normal tissues by placing an essential gene, *asd*, under the control of a hypoxia-induced promoter. The *asd* gene of *Salmonella* encodes an enzyme essential for the synthesis of Diaminopimelic acid (DAP), which is itself an essential component of the bacterial cell wall and not present in mammalian systems. With *asd* expressed only in hypoxic conditions the bacterium is able to grow readily under hypoxia, but will lyse under normal growth conditions^[12].

Bifidobacterium infantis

Bifidobacterium Infantis is a kind of Bifidobacteria that is non-pathogenic and anaerobic, and thus they can selectively localize and proliferate in the hypoxic environment in several types of solid tumors after systemic application^[24]. The fms-like tyrosine kinase receptor (Flt) is a transmembrane receptor of the tyrosine kinase family, which has been identified as a receptor for VEGF. It has an important role in tumor growth and metastasis, and are associated with poor prognosis in clinical human tumors. First identified in 1993, the soluble Flt (sFlt-1) was immediately found to exert powerful antiangiogenesis function. As the soluble form of extracellular part of VEGF receptor-1 (VEGFR-1), sFlt-1 can compete with VEGFR-1 for VEGF and exert its function. It has been confirmed that sFlt-1 can suppress both the growth and metastasis of solid tumor by many investigations^[16].

Bifidobacterium Infantis-mediated sFlt-1 gene

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transferring system was successfully constructed and could express sFlt-1 at the levels of gene and protein. This system could significantly inhibit growth of HUVECs induced by VEGF *in vitro*. Moreover, it could inhibit the tumor growth and prolong survival time of LLC C57BL/6 mice safely. This may be a promising therapeutic strategy for cancer patients.

Lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) are beneficial probiotic organisms that contribute to improved nutrition, microbial balance, and immuno-enhancement of the intestinal tract, as well as lower cholesterol. Although present in many foods, most trials have been in spreads or dairy products^[14].

Weissella cibaria

Weissella is a newly separated lactic acid bacteria of lactobacillus family that was identified by a recent DNA technique. It is a Gram-positive and catalase negative bacteria included in generally recognized as safe. Studies have reported that microorganisms, including lactic acid bacteria, secreted exopolysaccharides (EPS) with the anticancer, anti-inflammatory, immune modulating, and blood cholesterol declining functions^[1].

Lactobacillus plantarum

Lactobacillus genus is a microorganism which does not form spores and is an anaerobic and facultative anaerobic gram-positive bacterium. This bacterium is not only widely dispersed in nature but is also found in human oral cavity and digestive organs. This bacterium is a beneficial microorganism that is widely used as a starter for various fermented dairy products

L. plantarum, isolated from kimchi, also exhibited a strong antimutagenic effect against *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, 4-Nitroquinoline-1-oxide. Furthermore, *L. Plantarum*, had stronger anti-

mutagenic effects. The macrophagocytes provided with *L. plantarum* strengthened phagocytosis, and displayed anticancer effects in ascites carcinoma and solid tumor due to the polysaccharide chains of muramic acid in the *L. plantarum*'s cell wall. Among lactic acid bacteria's cell wall substances, polysaccharide types rather than glycopeptide play a pivotal role in cancer suppression^[18]

Daily consumption of Bifidobacterium spp (B. longum SPM1207) can help in managing mild to moderate hypercholesterolemia, with potential to improve human health by helping to prevent colon cancer and constipation.

Escherichia coli nissle

Facultative anaerobic bacteria like *E. coli* can colonize solid tumors often resulting in tumor growth retardation or even clearance. Little mechanistic knowledge is available for this phenomenon which is however crucial for optimization and further implementation in the clinic.

Escherichia coli Nissle 1917 may be developed as an orally administered diagnostic that can noninvasively indicate the presence of liver metastasis by producing easily detectable signals in urine. Our microbial diagnostic generated a high-contrast urine signal through selective expansion in liver metastases (10(6)-fold enrichment) and high expression of a lacZ reporter maintained by engineering a stable plasmid system. The lacZ reporter cleaves a substrate to produce a small molecule that can be detected in urine^[4].

Butyrate

Butyrate as an important short chain fatty acid has been shown to affect different kinds of cancer cells. Butyrate exerts its anti-cancerous effects by several mechanisms and has lead to successful outcomes in phase I and II clinical trials. Moreover, since solid tumors grow rapidly, multiple regions of hypoxia and anoxia forms within them that provide

TABLE 1 : Differences between Ad/mβG and *E. coli* (lux/βG)^[9]

Therapy	Ad/mβG	<i>E. coli</i> (lux/βG)
Tumor distribution	Live cells	Border between live cells and necrotic areas
Enzyme location	Cancer cell surface	<i>E. coli</i> periplasmic space
Enzyme source	Mouse beta-glucuronidase	<i>E. coli</i> beta-glucuronidase

good niches for the growth of anaerobic bacteria. It has been shown that bacterial tumor targeting is an applicable strategy for tumor-selective therapy^[20].

Sodium butyrate has an effect on AR coregulators expression, transcription activity and histone acetylation in cancer cells, but there is only minimal effect in normal cells. In addition, the results of changes in acetylation level on lysine residues of histone H4 after sodium butyrate treatment confirm its epigenetic effect on prostate cancer cells^[15].

Although the underlying mechanisms by which butyrate regulates cell proliferation and/or differentiation are not fully understood, it has been shown that butyrate action could involve various effects on gene expression, which are often attributed to its capacity to act as an inhibitor of histone deacetylases (HDACs). This effect leads to a hyperacetylation of histones and increased accessibility of transcription factors to promoters in the DNA^[11].

Moreover, butyrate influences post-translational modifications including DNA methylation, histone methylation, histone phosphorylation and hyperacetylation of nonhistone proteins. Those diverse effects may explain the impact of butyrate on the expression of key regulators of apoptosis and cell cycle such as the proapoptotic protein BAK12 and the cell cycle proteins, cyclins D and p21^[13].

Compelling recent evidence suggests that the metabolic profile of cancer cells is a critical determinant of their proliferative properties. Since butyrate treatment is expected to impact cellular metabolic pathways, we hypothesized that butyrate could also exert its antiproliferative properties by altering cellular metabolism and more precisely by regulating the expression of metabolic enzymes^[2].

During tumorigenesis, most cancer cells exhibit an altered metabolism that is characterized by elevated glucose uptake and an increased glycolytic rate leading to high lactate release; this metabolic phenotype known as the Warburg effect constitutes a major feature of aggressive tumors^[19].

Nonpathogenic anaerobic butyrate-producing bacteria may be a versatile tool in tumor therapy as they can grow in anoxic and hypoxic regions of tumors and influence tumor cells. So, tumor targeting with nonpathogenic anaerobic bacteria with a higher

capacity to produce butyrate could be the focus of future research.

CONCLUSION

Bacteriotherapy as mentioned above will be the most safe and selective treatment of cancer in the future especially with the development of biotechnology techniques. Acknowledgements:

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ABBREVIATIONS

- Prodrug converting enzyme (PCE)
- Clostridium-Directed Enzyme Prodrug Therapy (CDEPT)
- Nitroreductase (NTR)
- Diaminopimelic acid (DAP)
- Fms-like tyrosine kinase receptor (Flt)
- vascular endothelial growth factor (VEGF)
- Bacteria directed enzyme prodrug therapy (BDEPT)
- Exopolysaccharide (EPS)

REFERENCES

- [1] S.K.Cha, B.H.Ahn, J.R.Kim; Korea food research institute, Assignee Weissella cibaria 148-2 lactic bacteria for functional healthy effect and Makgeolli containing the same, Korea patent KR 1020080133488.24, inventors, (2008).
- [2] H.Chirakkal, S.H.Leech, K.E.Brookes, A.L.Prais, J.S.Waby, B.M.Corfe; (2006).
- [3] Upregulation of BAK by butyrate in the colon is associated with increased Sp3 binding, *Oncogene*, **25**, 7192–200.
- [4] T.Danino, A.Prindle, G.A.Kwong, M.Skalak, H.Li, K.Allen, J.Hasty, S.N.Bhatia; Programmable probiotics for detection of cancer in urine, *Sci.Transl.Med.*, **27**, 7(289):289ra84 (2015).
- [5] J.Drees, M.Mertensotto, G.Liu, J.Panyam, A.Leonard, L.Augustin, J.Schottel, D.Saltzman; Attenuated *Salmonella enterica* Typhimurium reduces tumor burden in an autochthonous breast cancer

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- model, *Anticancer Res.*, **35(2)**, 843-9 (2015).
- [6] M.J.Fonseca, J.C.Jagtenberg, H.J.Haisma, G.Storm; Liposome-mediated targeting of enzymes to cancer cells for site-specific activation of prodrugs: Comparison with the corresponding antibody-enzyme conjugate, *Pharm Res.*, **20**, 423–428 (2003).
- [7] J.T.Heap, J.Theys, M.Ehsaan, A.M.Kubiak, L.Dubois, K.Paesmans, L.V.Mellaert, R.Knox, S.A.Kuehne, P.Lambin, N.P.Minton; Spores of *Clostridium* engineered for clinical efficacy and safety cause regression and cure of tumors in vivo, *Oncotarget*, **15**, **5(7)**, 1761-9 (2014).
- [8] Hoffman RM Zhao M; Methods for the development of tumor-targeting bacteria *Expert Opin Drug Discov*, **9(7)**, 741-50 (2014).
- [9] Y.T.Hsieh, K.C.Chen, C.M.Cheng, T.L.Cheng, M.H.Tao, S.R.Roffler; Impediments to enhancement of CPT-11 anticancer activity by *E. coli* directed beta-glucuronidase therapy, *PLoS One*, **17**, 10(2):e0118028 (2015).
- [10] K.K.Jain; Use of bacteria as anticancer agents, *Expert Opin.Biol. Ther.*, **1(2)**, 291-300 (2001).
- [11] Jean-Marc Blouin, Graziella Penot, Martine Collinet, Magali Nacfer, Claude Forest, Pierre Laurent-Puig, Xavier Coumoul, Robert Barouki, Chantal Benelli, Sylvie Bortoli; Butyrate elicits a metabolic switch in human colon cancer cells by targeting the pyruvate dehydrogenase complex, *Int.J.Cancer*, **128**, 2591–2601 (2011).
- [12] R.W.Kasinskas, N.S.Forbes; *Salmonella typhimurium* specifically chemotax and proliferate in heterogeneous tumor tissue in vitro, *Biotechnol Bioeng*, **94**, 710–721 (2006).
- [13] H.Kobayashi, E.M.Tan, S.E.Fleming; Sodium butyrate inhibits cell growth and stimulates p21WAF1/CIP1 protein in human colonic adenocarcinoma cells independently of p53 status, *Nutr Cancer*, **46**, 202–11 (2003).
- [14] Lee do K1, S.Jang, E.H.Baek, M.J.Kim, K.S.Lee, H.S.Shin, M.J.Chung, J.E.Kim, K.O.Lee, N.J.Ha; Lactic acid bacteria affect serum cholesterol levels, Harmful fecal enzyme activity, and fecal water content, *Lipids Health Dis.*, **11**, 8:21 (2009).
- [15] Lenka Paskova, Katerina Smesny Trtkova, Barbora Fialova, Andrea Benedikova, Katerina Langovad Zdenek Kolar; Different effect of sodium butyrate on cancer and normal prostate cells, *Toxicology in Vitro*, **27(5)**, 1489–1495 (2013).
- [16] J.Liu, J.Li, C.Su, B.Huang, S.Luo; Soluble Fms-like tyrosine kinase-1 expression inhibits the growth of multiple myeloma in nude mice, *Acta Biochim.Biophys.Sin.*, **39**, 499–506 (2007).
- [17] N.Nair, T.Kasai, M.Seno; Bacteria: Prospective savior in battle against cancer, *Anticancer Res.*, **34(11)**, 6289-96 (2014).
- [18] C.Rhee, H.Park; Culture conditions on the mutagenic effects of *Lactobacillus plantarum* KLAB21 isolated from kimchi against N-methyl-N0-nitro-N-nitrosoguanidine and 4-nitroquinoline-1-oxide, *Korean J.Food Sci.Technol.*, **150**, 417–23 (2000).
- [19] S.Siavoshian, J.P.Segain, M.Kornprobst, C.Bonnet, C.Cherbut, J.P.Galmiche, H.M.Blottiere; Butyrate and trichostatin A, effects on the proliferation/differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression, *Gut*, **46**, 507–14 (2000).
- [20] H.Tirandaz, E.Mohammadi; Efficient tumor targeting by anaerobic butyrate-producing bacteria, *Med.Hypotheses*, **80(5)**, 675-8 (2013).
- [21] R.L.Warren, D.J.Freeman, S.Pleasance, P.Watson, R.A.Moore, K.Cochrane, E.Allen-Vercoe, R.A.Holt; Co-occurrence of anaerobic bacteria in colorectal carcinomas, *Microbiome*, 2013 May 15, **1(1)**, 16 (2013).
- [22] Yu B1, M.Yang, L.Shi, Y.Yao, Q.Jiang, X.Li, L.H.Tang, B.J.Zheng, K.Y.Yuen, D.K.Smith, E.Song, J.D.Huang; Explicit hypoxia targeting with tumor suppression by creating an “obligate” anaerobic *Salmonella Typhimurium* strain, *Sci.Rep.*, **2**, 436 (2012).
- [23] Y.Zhang, N.Zhang, R.M.Hoffman, M.Zhao; Comparison of the selective targeting efficacy of *Salmonella typhimurium* A1-R and VNP20009 on the lewis lung carcinoma in nude mice, *Oncotarget*, (2015).
- [24] Zhu H1, Z.Li, S.Mao, B.Ma, S.Zhou, L.Deng, T.Liu, D.Cui, Y.Zhao, J.He, C.Yi, Y.Huang; Antitumor effect of sFlt-1 gene therapy system mediated by *Bifidobacterium Infantis* on Lewis lung cancer in mice, *Cancer Gene Ther.*, **18(12)**, 884-96 (2011).