THERAPEUTIC APPROACH TOWARDS COLON CANCER: BACTERIAL AID TO THE RESCUE.

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ABSTRACT

The past years have witnessed the inculcation of interest in scientists regarding colon cancer treatment with microorganisms. This has been due to the observations that some microbes prefer to replicate selectively or exhibit preferential accumulation within tumor microenvironment. Besides, conventional therapies implied in this field displays resistance to the disease at some point of time. The preferential replication of microorganisms provides immense probability to amplify the therapeutic effect of the microorganism while sparing the normal tissues from toxicity. With the presence of plentiful effector genes which could be engineered into bacterial hosts, the therapies could be comprehensive to sequential or concurrent administration of similar or dissimilar bacteria containing different gene products. Demonstration of the concept of selective intratumoral accumulation of bacteria in cancer patients can be anticipated to lead to an enormous and new repertoire of therapeutic options for the treatment of metastatic disease such as colon cancer.

Keywords: Colon cancer, bacteria, *Escherichia coli*, hemolysin, immunotherapy.

INTRODUCTION

Colorectal cancer is a frequent malignancy in developed countries and attains the 3rd rank for common cancer in the United States [1]. More than 80% of it occurs sporadically [2]. Though the risk to acquire colorectal cancer is mainly influenced by lifestyle, about 20 to 30 % of all cases can be attributed to genetic predisposition [3]. However, colorectal cancer exhibits a low incidence in under-developed countries [4]. This imbalance related to geographical regions suggests a contribution of the environmental factors to the resistance of endemic populations to intestinal neoplasia [5]. Even though the epidemiology of colon cancer stays poorly understood, yet it can be said that there is an inverse relationship between diarrhea related countries and the occurrence of colon cancer. Experimental colon cancer treatments are anticipated to treat cancer by bringing about an improvement, supplementation or replacement of conventional methods, which include photodynamic therapy, gene therapy, hyperthermia therapy, telomerase therapy, dichloroacetate, non-invasive RF cancer treatment, diet therapy, complementary and alternative therapy, insulin potentiating therapy and bacterial treatment [6]. However, many of these therapies lead to controversies owing to lack of substantiation, practicability, availability, effectiveness, specificity and selectivity. It has been reported that some microorganisms show selective replication in tumor cells or preferential accumulation in the tumor micro-

environment which offers a great prospective for cancer therapy. Hence researchers battling colon cancer have found a new means to tackle the problem by using bacteria and its products as effective weapons. This review brings forward the usage of bacteria as a novel therapy for colorectal cancer.

BACKGROUND OF BACTERIAL USAGE FOR CANCER THERAPY

The use of bacteria or their extracts in the treatment of cancer can be traced back to more than hundred years when the American physician William Coley noticed that one of his patients suffering from neck cancer began to recover following an infection with *Streptococcus pyogenes* infection. His assembled thoughts and well-planned ideas gave him the inspiration to use bacteria and their toxins to cure end stage cancers. Problems with the predictability of patient responses made him develop a safer vaccine in the late 1800's which constituted of two killed bacterial species, *S. pyogenes* and *Serratia marcescens* to simulate an infection with the accompanying fever without having the risk of an actual infection [7,8]. The vaccine was extensively used to fruitfully treat sarcomas, carcinomas, lymphomas, melanomas and myelomas. Complete, prolonged regression of advanced malignancy was found in many cases [9]. 'Coley's toxins' which were toxic bacterial derivatives, were also thoroughly investigated for potential anticancer activity [10]. The early success of Coley's toxins gave way to the footholds for current advances in this field. Even in the early years of 1944, the endotoxin of *Serratia marcescens* was shown to be the "hemorrhage-producing factor" which caused tumor regression [11]. Many subsequent reports have displayed the varying efficacy of several detoxified bacterial preparations of LPS [12, 13, 14], with or without any additional components, including some LPS preparation vaccines of *Pseudomonas aeruginosa* [15]. These provided noteworthy persistence of remission and survival in patients with acute myelogenous leukemia compared to patients not treated with the LPS. All these implementations of past years motivated the scientists for developing efficacious treatment alternatives for colon cancer.

BACTERIAL PROPERTIES THAT MAKE THEM SIGNIFICANT THERAPEUTIC ALTERNATIVES

Bacteria colonize in host cells after binding, in a discerning manner. Some species of bacteria prefer to replicate within tumor cells. Besides, bacteria are motile, which facilitates them to spread throughout the tumor and can aid to target systemic disease. Because of their large genomic size, bacteria can easily express multiple therapeutic transgenes, such as cytokines or pro–drug-converting enzymes, and their spread can be regulated and kept under control with antibiotics if necessary. [16]. It is believed that bacteria have the capability to activate the immune system on entering the host cells and then allow regression of cancer cells. The proliferation of anaerobic bacteria specifically within tumors is a well-proven fact [17, 18, 19]. Coley's observations provided a new ground for the scientists who discovered that certain species of anaerobic bacteria, especially those belonging to the genus *Clostridium*, consume oxygen-poor cancerous tissue and expire when they come into contact with the oxygenated part of the tumor. This makes them safe for the rest of the body parts [20]. These findings lay the foundation of the rationale to utilize bacteria for cancer therapy.

ETEC INFECTIONS VS. COLON CANCER

Enterotoxigenic Escherichia coli (ETEC) produce heat-stable enterotoxins (STs), one of the major causes of secretory diarrhea in endemic populations, travelers and animal herds employed for agricultural purposes [21, 22]. These enterotoxins are small peptides of about 19 amino acids and encoded by plasmids. They bind to guanylyl cyclase C (GC-C), particularly expressed in intestinal epithelial cells [23, 24]. Involvement of STs

with the extracellular domain of GC-C activates the intracellular catalytic domain thus converting GTP into cGMP [25, 26]. The second messenger cGMP then activates cGMP-dependent protein kinase (PKG) II, the obvious downstream effector for this cyclic nucleotide, which as a consequence, results in secretory diarrhea [25- 27]. By this process, STs show molecular mimicry in which enterotoxigenic bacteria develop a strategy for propagation, thus exploiting the normal intestinal physiology. STs are homologous to the endogenous peptides guanylin and uroguanylin both structurally and functionally [28, 29], mediating autocrine/ paracrine control of intestinal fluid and electrolyte homeostasis [30]. GC-C and its ligands are also associated with the regulation of the balance of proliferation and differentiation along the crypt-to-villus axis in the intestine, apart from volume homeostasis [31]. The expression of endogenous GC-C ligands is generally lost during tumorigenesis. The subsequent loss of signaling represents a key mutational event that leads to neoplastic transformation in the colon [32- 34]. Drawing from these observations, Pitari *et al* [35] demonstrated that a bacterial heat-stable enterotoxin (ST) could suppress colon cancer cell proliferation by a guanylyl cyclase C-mediated signaling cascade. The heat-stable enterotoxin suppressed the proliferation by increasing intracellular cGMP. This effect was mimicked by the cell permeant analog 8-br-cGMP. The antiproliferative effects of the enterotoxin and 8-br-cGMP were reversed by cyclic nucleotide-gated channel inhibitor, as well as by the removal of extracellular Ca2+, or chelation of intracellular Ca2+. In fact, both the enterotoxin and 8-br-cGMP promoted Ca2+ influx and inhibition of DNA synthesis in colon cancer cells. In other studies, it was reported that *E. coli* STa causes IP3 mediated calcium mobilization from intracellular stores and other signaling molecules in COLO-205 colon carcinoma cell line [36, 37], which can be attributed to anti-proliferative effect of this toxin on colon carcinoma cells. Besides, it was also evidenced by Saha *et al.* [38] that E. coli STa inhibits the proliferation of the colonic carcinoma cell line COLO-205 by the PKG-ERK44/42 mediated pathway.

ROLE OF HEMOLYSIN IN COLON CANCER

In a recent study, it has been shown by Chowdhury *et al.* [39] that thermostable direct hemolysin (TDH) secreted by *Vibrio parahaemolyticus* has anti-proliferative effect on COLO-205 colon carcinoma cell line. Several studies reveal that TDH induces diarrhea by elevating the intracellular calcium through activation of calcium influx in intestinal epithelial cells [40–42]. It is well known that calcium ion ($Ca^{2}+$) is a universal secondary messenger and a key player in many cellular signal transduction pathways [43, 44]. A lot of reports have shown that $Ca^{2}+$ plays a crucial role in prevention of colon carcinogenesis [45, 46]. Ca2+ opposes tumorigenesis by restricting proliferation through promotion of E-cadherin expression and inhibition of β- catenin/Tcf-4 signaling [47, 48]. As TDH causes an increase in intracellular calcium level in intestinal epithelial cells through activation of calcium influx from extracellular environment and calcium sensing receptor plays a vital role in influx of extracellular calcium, hence the potential of TDH in the down-regulation of colonic carcinoma cell proliferation (COLO-205) was evaluated. The downregulation occurred mainly through the involvement of E-cadherin-β-catenin mediated pathway and the inhibition of cell cycle regulators along with the upregulation

of cell cycle inhibitors.

LACTIC ACID PRODUCING BACTERIA AND COLON CANCER

The lactic acid producing bacteria which are used for fermenting milk and other dairy products have anti-mutagenic and anti-carcinogenic properties [49-51]. It was found that dietary intake of *Lactobacillus acidophilus* suppressed DMH-induced colon tumors

and increased the latency of tumors in rodents [52]. Singh *et al*. [53] investigated the role of *Bifidobacterium longum*, a lactic acid producing enterobacterium, on the cell proliferation of tumors in male F344 rats. Their data demonstrated that dietary administration of lyophilized cultures of *B. longum* resulted in significant suppression of colon tumor incidence, tumor multiplicity and tumor volume. The precise mechanisms by which lactic acid bacteria inhibits colon cancer may be presently unknown. However, such mechanisms might include enhancement of the host's immune response, binding and degradation of potential carcinogens, quantitative and qualitative alterations in the intestinal microflora incriminated in producing putative carcinogens and promoters, formation of antitumorigenic or antimutagenic compounds in the colon, changing the metabolic activities of intestinal microflora, alteration of physicochemical conditions in the colon and affecting the physiology of the host [54].

IMMUNOTHERAPY OF COLON CANCER USING BACTERIA

Immunotherapeutic approach employs the stimulation of immune system to destroy cancer cells. Hence bacteria can be used to enhance the antigenicity of tumor cells [55]. For example, preliminary studies by Hoover *et al*. [56] suggested that active specific immunotherapy (ASI) of colon cancer using autologous tumor cell vaccines had the capacity to improve recurrence-free interval and survival. The vaccine endeavored to stimulate the host's immune defenses against tumor-associated antigens by enhancing the immunogenicity of the patient's own tumor cells with an immunomodulating adjuvant, such as Bacillus Calmette-Guérin (BCG). In 2005, Uyl-de Groot *et al*. [57] conducted a multicenter, randomized controlled phase III clinical trial with Stage II and III colon cancer patients using ASI. Autologous tumor cells were used with the immunomodulating adjuvant Bacillus Calmette-Guérin (BCG) in a vaccine (OncoVAX®). Patients were randomly made to receive either OncoVAX® or no therapy after surgical resection of the primary tumor. Analysis of prognostic benefit with a 5.8 year median follow- up, showed that the beneficial effects of OncoVAX® were statistically significant at all endpoints including recurrence-free interval, overall survival, and recurrence-free survival in Stage II colon cancer patients. Regrettably, no statistically significant prognostic benefits were obtained in Stage III patients. Recently it has been found out that BCG/ CWS has a radiosensitizing effect on colon cancer cells by inducing autophagic cell death. Both *in vitro* and *in vivo* studies have proved that combining BCG/ WCS with ionizing radiation can be used as an effective therapeutic approach [58].

USE OF ENGINEERED BACTERIA FOR COLON CANCER THERAPY

Attenuated bacteria enhance the stimulation of the innate immune system and increase the safety of a vaccine [59]; therefore they may be ideal for the delivery of vaccines. Dang *et al*. [60] used mitomycin C, a chemotherapeutic agent and dolastatin-10, an antivascular agent along with the spores of an attenuated anaerobic bacterium, *Clostridium novyi*, to treat colorectal cancer cells. The rationale behind this combined therapy, also known as combination bacteriolytic therapy or COBALT, was that, while the anaerobic bacteria dwelt in the anaerobic zone of the tumor cores, the antivascular agent (dolastatin-10) would provide an extensive hypoxic area for the growth of bacteria and starve the tumors of oxygen and essential nutrients. The chemotherapeutic agent, mitomycin C, helped to attack the tumor cells in the well-perfused, non-necrotic outside cells of the tumors, leading to their total obliteration [60, 61]. The results of this kind of study were highly impressive. In the absence of the bacteria, but in the presence of mitomycin C and dolastatin-10, the tumors exhibited persistance for a much longer time and showed limited regression, while including the bacteria led to

widespread desertion of the tumors within a short stretch of time. In certain cases, complete dissolution of the tumors was evidenced, thus leaving the animals free of tumor. Chou *et al.* [62] showed that vaccination with an attenuated *S. typhimurium* oral DNA vaccine carrying the α-fetoprotein gene could be a promising stategy to prevent hepatocellular carcinoma and colon cancer. Recently it was reported that live and highly attenuated *Listeria monocytogenes*-based vaccine delays tumor growth in Her2/neu transgenic animals, which supports the future clinical development of this vaccine for the treatment of Her2/neu overexppressing malignancies, such as colorectal and pancreatic cancer [63].

DRAWBACKS OF BACTERIAL THERAPY

The main drawback of using bacteria for cancer therapy is that they are quite toxic in nature and if the toxicity is removed, then it will not be an efficient candidate for cancer treatment. In Dang's work [60] it was seen that even after removal of toxicity in attenuated *Clostridium novyi*, the mortality of mice was around 45%. Besides, bacteria do not seem to devour the whole of the malignant tissue which requires the usage of chemotherapy along with bacterial therapy. In case of using attenuated bacteria the main problem is the inaccessibility because it demands intratumoral injection [64]. Then there is also the probability of DNA mutation which may cause loss of functionality or overstated infection in the tumor area. Hence there is a necessity to go through the present problems and pave a path for better development measures which can employ bacteria for cancer therapy.

CONCLUSION

Recent research has uncovered a great deal of information in using bacteria for the cure of colon cancer. Factors which suggest a protective role of a bacterial species include: (1) colonization lowers the risk of a certain cancer; (2) introduction of the bacteria or its toxins cures colon cancer. Successful treatment for cancers was reported by Dr. Coley and others one hundred years ago. His approach of using killed bacterial vaccines was astonishingly very effective in some patients even in the latest stages of cancer. Dr. Coley believed that the human immune system had the power to cure cancers if properly stimulated. Today, some investigators see eye to eye with his belief and have designed new treatment technologies that stimulate the immune system to identify and target the lesion. Live, attenuated bacteria as antitumor agents and vectors for gene directed enzyme prodrug therapy have come up as potential strategies for colon cancer cure. Investigation is being carried out using chimeric toxins as future toxin-based anticancer therapies. These studies have reported successful treatment of certain cancers and prevention of recurrences. The continued exploration in this field will be able to bring research closer to the prevention, early diagnosis and justly efficient treatment of this bane of mankind.

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