Review

Microbial-Based Therapy of Cancer
A New Twist to Age Old Practice

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ABSTRACT
The use of bacteria in the regression of tumors has long been known. Various approaches for using bacteria in cancer therapy include the use of bacteria as sensitizing agents for chemotherapy, as delivery agents for cancer drugs and as agents for gene therapy. The tumor regression stimulated by infecting microorganisms has been attributed to activation of the immune system of the host. However, recent studies indicate that when tumor-harboring mice with defective immune systems are infected with certain microorganisms, the regression of the tumor is still observed, suggesting that there are other host factors contributing to the microbial associated regression of tumors. Since the use of live or attenuated bacteria for tumor regression has associated toxic effects, studies are in progress to identify a pure microbial metabolite or any component of the microbial cell that might have anti-cancer activity. It has now been demonstrated that a redox protein from Pseudomonas aeruginosa, a cupredoxin, can enter into human cancer cells and trigger the apoptotic cell death. In vivo, this cupredoxin can lead to the regression of tumor growth in immunodeficient mice harboring xenografted melanomas and breast cancer tumors without inducing significant toxic effects, suggesting that it has potential anti-cancer activity. This bacterial protein interacts with p53 and modulates mammalian cellular activity. Hence, it could potentially be used as an anti-cancer agent for solid tumors and has translational value in tumor-targeted or in combinational-biochemotherapy strategies for cancer treatments. Here, we focus on diverse approaches to cancer biotherapy, including bacteriolytic and bacterially-derived anti-cancer agents with an emphasis on their mechanism of action and therapeutic potential.

INTRODUCTION
In general the growth of tumors or tumor kinetics depends on several closely related factors:
1. Cell cycle time, or the average time for a mitotically dividing cell. This determines the maximum growth rate of a tumor.
2. Growth fraction, or the fraction of cells undergoing cell division.
3. The total number of cancer cells in the population, which is an indicator of total cancer burden. As the number of cancer cells increases, so does the number of resistant cells, which leads to reducing the effectiveness of an anti-tumor agent.

Variations in these factors are responsible for the variable rates of tumor growth observed in different tumors. Tumors grow most rapidly at small volumes. As they become large, growth slows down based on a complex process dependent on cell loss and tumor blood and oxygen supply. In order to have the best chances for a cure, anti-tumor agents must be given in a manner in which they can achieve a fractional cell killing in a logarithmic fashion.

Current strategies for cancer therapy are based on radiation treatment and the use of drugs. However, most solid tumors contain poorly vascularized areas characterized by hypoxia or anoxia, and these areas are relatively less sensitive to radiation in the absence of oxygen. Moreover, since drugs are transported by blood, the delivery of chemotherapeutic agents is more difficult in poorly vascularized tumors. Despite major scientific and technological progress in combinatorial chemistry, drugs derived from natural products still make an enormous contribution to drug development today.

Microorganisms have an inexhaustible metabolic potential and can adapt to various environmental conditions. Tumors with a metabolically compromised microenvironment provide a haven for a number of anaerobic bacteria. In the past several years, many strains of facultative and obligate anaerobic bacteria have been shown to localize and cause lysis in transplanted tumors in animals.1-3 There are several reports suggesting a correlation
between bacterial infection and tumor regression. To our knowledge the first scientific report of tumor shrinkage due to therapeutic infection was published in Berlin, Germany, in 1868.4 Some years later in the United States, William Coley5 discovered that many cancer patients could be cured of advanced metastatic cancer by repeated infection or injection of bacterial extracts consisting of gram-positive Streptococcus pyogenes alone or in combination with gram-negative Serratia marcescens. Since then, a number of studies have suggested a direct correlation between various live/attenuated bacteria or their components and the regression of tumors. An ideal anti-cancer bacterium should be nontoxic to the host, only able to replicate in the tumor, able to disperse evenly throughout the tumor, be nonimmunogenic and have the ability to cause lysis of the tumor. A significant progress has been made on each of these points. Bacteria were tested as nonspecific immuno-stimulators to boost immunity against cancer. A variety of test models were used in such studies, which include sarcoma, rhabdomyo-sarcoma, carcinoma, melanoma and breast cancer. The bacteria that were tested for in vivo regression of solid tumors include: Clostridium (obligatory anaerobes such as C. histolyticum, C. tetani, C. butyricum, C. acetobutylicum, C. pectinovorum, C. tyrobutyricum, and C. novyi); Bifidobacterium (obligatory anaerobes including B. bifidum, B. infantis and B. longum); Salmonella typhimurium (a facultative anaerobe) and Clostridium parvum (an obligate anaerobe). B. bifidum is an exception in that it is a useful part of the microflora of the human intestine and is used to prepare bacterial medicine and fermented milk products. In these experimental studies, some test animals with malignant tumors completely recovered, while regression of tumor growth occurred in other animals. It has been demonstrated previously, that a protozoan, Toxoplasma gondii, when injected into melanoma-bearing mice, caused tumor regression by blocking angiogenesis. It was assumed that the parasitic infection induced the release of anti-angiogenic soluble factors that created hypoxic conditions in the tumor and led to necrosis. Modern molecular biology techniques can be used to manipulate bacteria for efficient and safe use in tumor therapy. One such example comes from studies by Dang et al.7 who used heat shock to eliminate lethal toxin genes from Clostridium novyi and then used such an attenuated bacterium in combination with the chemotherapeutic agent mitomycin C and the anti-vascular agent dolastatin-10 to treat colorectal cancer. The rationale behind this combined therapy (called combination bacteriolytic therapy, or COBALT) was that the anaerobic bacteria grew in the anaerobic zone within tumor cores; the anti-vascular agent would create more extensive hypoxic areas for bacterial growth, thereby depriving the tumors of oxygen and essential nutrients while the chemotherapeutic agent attacked and destroyed the remaining well-perfused tumor cells. The results of this study were highly significant: in the absence of bacteria, but in the presence of mitomycin C and dolastatin -10, the tumors persisted for a much longer time and showed limited regression, while inclusion of bacteria led to extensive disappearance of the tumors within a short period of time and, in some cases, complete tumor dissolution that left the animal tumor-free. The major limitation of this study was its associated toxicity: 15–45% of the mice died within few days of beginning treatment, presumably due to the release of highly toxic metabolic products from the disintegrating tumors. Others and we have demonstrated the up-regulation of survivin and angiogenic factors induced by Helicobacter pylori in gastric cancer as well as in solid tumors and their surrounding areas, suggesting the importance of survivin for the inhibition of apoptosis in the pathogenesis of gastric cancer.8,10 The intra-tumor plasmid gene therapy with survivin antagonists could block the expression of survivin and angiogenic factors could enhance the anti-tumor activity.11 The expression of survivin in gastric cancer was associated with reduced apoptosis and COX-2 expression.

This review will describe how an in-depth knowledge of proteomics and biochemistry, coupled with an understanding of the p53 pathway, is allowing the development of novel strategies to manipulate the therapeutic potential of bacterial products. The use of pure bacterial products might help to lower the risk factors associated with the use of live/attenuated bacteria. Work in this field represents an outstanding illustration of how basic scientific research can be translated into clinical applications for cancer therapy and the many challenges involved.

**ADJUVANT CANCER THERAPY WITH BACTERIA**

Despite setbacks and inconsistent data, the work with bacterial extracts led to the approval of Bacillus Calmette-Guerin (BCG), which is widely used for the local treatment of bladder cancer.12 Several studies have shown a clear relationship between the use of BCG immunoprophylaxis after surgical removal of the tumor and a decreased recurrence rate or delayed period during which recurrence could occur.13,14 The mechanism of anti-cancer activity of BCG has been attributed to its effect on the immune system, with CD4 and CD8 T lymphocytes playing a major role.15 In addition to its use as an immunoprophylactic agent, BCG has been widely used as an adjuvant for modified cancer cell vaccines for several different types of tumors including central nervous system (CNS) malignancies. BCG is also included as an immunoadjuvant for CancerVaxTM (Cancervax Corp., CA, USA), a polyvalent tumor cell vaccine that has been shown to improve overall survival in stage II melanoma patients and in patients treated after curative resection of distant melanoma.16 On the other hand, BCG –induced production of interferon (IFN)-γ has been suggested to be the effector mechanism for the anti-tumor effect of BCG on melanoma.17 In addition, an in vitro human system was used to analyze the role of Natural Killer (NK) cells in BCG-induced cellular cytotoxicity: mononuclear cells were treated with BCG for seven days and BCG-activated NK cells significantly destroyed bladder tumor cells. Similarly, using C57BL/6 wild type mice, NK-deficient mice and mice treated with anti-NK-1.1 monoclonal antibody, Brandau et al.18 demonstrated that the use of BCG significantly prolonged survival in wild type mice. BCG therapy was completely ineffective in NK deficient mice or mice treated with Nk1.1 antibody. These studies suggest a key role for NK cells in BCG immunotherapy. BCG stimulation of the human innate immune system—as judged by altered levels of interleukin-12 (IL-12), IL-8, IL-10 and IFN-γ in the blood of patients with lung cancer—also demonstrated that BCG has a role in the modulation of the immune system.19 However BCG is effective predominantly in superficial cancers such as bladder cancer, and similar effects have not been observed in lung cancer or melanoma.21 Such differential effects may be attributed to cytokine network modulation or functional TLR receptor (Toll like receptors) repertoire expressed by surrounding tissues, recruited cells, or the nature of malignant cells themselves. Members of the toll like receptor gene family convey the signals induced by various bacterial factors such as lipopolysaccharides, lipoproteins, toxins, peptidoglycans and CpG nucleic acids, activates signal transduction pathways that result in transcriptional regulation and stimulate immune function. Because of their ability to modulate adaptive immunity, Toll like receptors represent strategic targets for
cancer and other diseases that involve inappropriate adaptive immune responses such as allergy.22

**LIVE ATTENUATED BACTERIA AS A VECTOR FOR CANCER TREATMENT**

With the advances in molecular biology, the scope of use of bacteria in cancer therapy has widened and various possibilities now include the use of bacteria as sensitizing agents for chemotherapy, as delivery agents for cancer drugs and as vectors for gene therapy. The most promising approaches are the use of genetically modified bacteria for selective destruction of tumors and bacterial gene-directed enzyme therapy.23 Live attenuated bacteria such as *Listeria* and *Salmonella* have shown great potential as a multivalent vaccine for the delivery of heterologous antigens.24,25 It has also been recently described that bacteria can act as delivery systems for DNA vaccines. Plasmids that encode antigens under the control of a viral or eukaryotic promoter can be incorporated into *Salmonella* or *Listeria*. Genetically improved attenuated strains of *Salmonella*, which can target tumor cells rather than normal cells at a ratio of 1000:1, have been used as vectors to introduce specific enzymes such as cytosine deaminase into tumors. Cytosine deaminase converts the nontoxic prodrug 5-fluorocytosine into the highly toxic anti-cancer drug 5-fluorouracil. Thus, injection of such attenuated *Salmonella* into mice harboring lung tumors, that were subsequently given injections of 5-fluorocytosine, has been reported to result in a significant reduction in tumor size. TAPET (Tumor amplified protein expression therapy) uses a genetically altered strain of *Salmonella* as a bacterial vector or vehicle to preferentially deliver an anti-cancer drug to solid tumor.23 More recently, bacterial strains, that specifically target tumors, such as *Bifidobacterium*, *Clostridium* and *Salmonella*, have been shown to preferentially replicate within solid tumors and have been used to transport and amplify genes encoding factors such as pro drug-converting enzymes, toxins, angiogenesis inhibitors and cytokines.26

**BACTERIA-DERIVED ANTI-CANCER AGENTS**

The normal mammalian cell cycle is composed of four phases. Cells that are committed to divide enter the G1 phase. Preliminary cellular processes are accomplished to prepare the cell to enter DNA synthesis—the S phase. Specific protein signals regulate the cell cycle and allow replication of the genome where the DNA content becomes tetraploid (4N). After completion of the S phase, the cell enters a second resting phase, G2, prior to undergoing mitosis. Then the cell progresses to the mitotic (M) phase, in which the chromosomes condense and separate leading to cell division, producing two daughter cells. Anti-tumor agents may be cell cycle specific or non-specific. Cell cycle non-specific anti-cancer agents, like alkylating agents have a linear dose-response curve; the number of cells killed increases linearly with the dose. However most of the bacteria-derived agents are cell cycle specific.

The use of live/attenuated bacteria in cancer therapy has a limited application because of associated toxic effects. Here, we will point to ways to achieve better effects with less toxicity by using a common bacterial component. Various microbial products have been used as anti-tumor agents; among these are members of the anthracycin, bleomycin, actinomycin, mitomycin and aureolic acid families. Clinically useful agents from these families are daunomycin-related agents, the peptolides (e.g., dactinomycin), the mitosanes (e.g., mitomycin C) and the glycosylated anthracenone mithramycin. These microbial derived anti-tumor agents have been described in a review by Rocha et al.27 Rhizoxin, a microbial metabolite containing more than one epoxide group, has been demonstrated to have anti-angiogenic activity and has anti-tubulin activity.28 The use of live microorganisms in cancer therapy has often been associated with unpredictable efficacy, a strong immune response, and associated side effects and toxicity. The human immune system is able to recognize bacterial DNA based on recognition of unmethylated cytidine-guanosine dinucleotides within a specific pattern of flanking bases (CpG motif).29,30 Such CpG motifs are potent inducers of deactivation and maturation of B cell and T cell responses.31 Hence, studies are in progress to identify pure metabolites of microbial origin or any component of the microbial cell, excluding DNA, that might have anti-tumor activity.

Some reports described the varying anti-tumor efficacy of certain detoxified bacterial LPS preparations with or without additional components,32 including some preparations of *Pseudomonas aeruginosa*.33 Bacterial lipid A induces various transcription factors via an intracellular signaling cascade. Its anti-tumor effect has been demonstrated in animals as well as in clinical trials.34 Lipotechoic acid (LTA) from *Streptococcus pyogenes* and *Enterococcus faecalis* has had a tumor protective effect both, alone and in combination with peptidoglycan of *Mesorhizobium catarhulis* and LPS from *E. coli*. This ability of LTA to enhance and/or amplify the anti-tumor mechanisms induced by primary stimuli may be related to its capability to trigger in vitro long-lasting anti-tumor activity in bone marrow macrophages.35,36 Some species of infectious microorganisms produce powerful immunostimulatory and disease-causing toxins, which are called super-antigens due to their ability to polyclonally activate large fractions of T-cell populations at very minute concentrations. The bacterial super-antigen Staphylococcal enterotoxin-A (SEA), produced by some strains of *Staphylococcus aureus*, induces proliferation of cytotoxic T lymphocytes and leads to cytokine production in vivo. Such super-antigens have been shown to be highly efficient for antibody-targeted therapy in various tumor models.37 The anti-tumor property of staphylococcal exotoxin-A is well documented in various tumors. A combination of protein A and super-antigen have an additive effect in prolonging the immune response in vivo and promoting a maximal anti-tumor effect. Another classical example is the identification of the secondary metabolites, epothilones (microtubule depolymerization inhibitors) of the myxobacterium *Sorangium cellulosum*, which have shown both in vitro and in vivo cytotoxicity in various cancer cells.38,39 Epothilones are anti-microtubule agents, and their mechanism of action is more or less similar to taxol. However, epothilones have several advantages over taxol. They are more water-soluble than taxol and most importantly, the epothilones are better able to inhibit the growth of tumor cells overexpressing the P-glycoprotein, a factor responsible for drug resistance in many tumors. Hence, epothilone-like compounds may be more useful than taxol in the treatment of multi-drug resistant tumors something which is problematic with taxol. Many analogues of epothilones have been synthesized such as Desoxepothilone B, which are more promising because of their efficacy against a range of tumors and relative lack of toxicity.40,41 Some studies have also demonstrated the role of the anthrax toxin as an anti-tumor agent. The anthrax toxin has a role in MEK signaling (MAP/ERK kinase) and offers a novel strategy to use in cancer treatment.42

The cell death induced by microorganism-derived substances raises an important question—what could be the mechanism of
mammalian cell death with whole microorganisms or their constituents, and can this cell death be differentially regulated in normal cells? The physiological mechanism involved is based on apoptosis. The intracellular location of the pathogen is not a prerequisite for the induction of apoptosis. The pathogen can inject toxic proteins into a target cell upon contact with it. This type of delivery of cytotoxic proteins requires cell-to-cell contact and is referred to as a type III secretion mechanism.\(^{43}\) It is used by *Pseudomonas aeruginosa* for the induction of apoptosis in macrophages and epithelial cells.\(^{54,45}\)

It has been recently reported that a bacterial redox protein, azurin, of *Pseudomonas aeruginosa* acts as an anti-cancer agent both in vitro and in vivo in a nude mouse model.\(^ {46}\) Azurin is a copper-containing oxidoreductase that is normally involved in the denitrification process in *P. aeruginosa*. Azurin is a member of a group of proteins collectively called cupredoxins, which are small, soluble redox proteins whose active site contains a type 1 copper protein. Azurin itself is involved in electron transfer during denitrification.

We have reported that purified azurin induces cytotoxicity in J774 macrophages, a sarcoma derived cell line, by somehow forming a complex with the tumor suppressor protein p53, thereby stabilizing it and enhancing its function as an inducer of pro-apoptotic activity.\(^ {47}\)

The redox activity of azurin is not important for its cytotoxic activity.\(^ {48}\) Azurin has been reported to induce apoptosis not only in macrophages but also in human melanoma cells (Mel 2), which harbor a functional tumor suppressor p53. The extent of apoptosis was much lower in the p53 null cell line Mel 6.\(^ {48}\) Azurin was shown to be internalized in Mel 2 cells and was localized predominantly in the cytosol and nuclear fraction. However in Mel 6 cells, azurin was localized primarily in the cytosolic fraction suggesting that intracellular trafficking of azurin to the nucleus may be p53-dependent.

The p53-tumor suppressor protein is a potent inhibitor of cell growth that induces cell cycle arrest and apoptosis\(^ {49}\) and mainly acts via transcriptional activity. p53 function is not required for normal cell growth and development of the cell; however, it plays an important role in preventing tumor development. It is activated by several types of stress signals and induces expression of several proteins that are involved in inhibition of cell proliferation or in promoting cell death by apoptosis. The importance of p53-induced apoptosis in tumor suppression is further evidenced by some tumor-derived p53 mutants that have a selective loss of function only for apoptosis and not for G1 arrest.\(^ {50}\) The choice of activation of particular target genes is controlled by covalent p53 modification and protein-protein interactions. Such interactions could make the difference between the life or apoptotic death of a cell.\(^ {51}\)

In the cell, p53 activity is tightly regulated and controlled by a negative feedback loop involving Mdm2 (murine double minute 2). Structurally and functionally, p53 consists of three well-defined regions, each having a specified function: The N-terminal region consists of a transactivation domain (amino acids 1–70) and a proline-rich stretch (amino acids 60–97). This region also contains an Mdm2 protein-binding site. The central region consists of a hydrophobic DNA binding domain (amino acids 94–312), a conserved region of p53 in various species. The C-terminal region consists of an oligomerization domain (amino acids 320–360) and a basic C-terminal domain (amino acids 360–393). In normal and nonstressed cells, the half-life of p53 is very short due to an autoregulatory feedback loop mechanism in which the Mdm2 protein plays a key role. This p53-Mdm2 auto-regulatory loop has provided a central target for therapeutic drug developments. Agents, that can alter the p53-Mdm2 pathway and protect p53 from Mdm2 degradation, are potential candidates for anticancer therapy. A recent study by Vassiliev et al.\(^ {52}\) is a classical example demonstrating that the use of such protein-protein interactions could be valuable in p53-based therapy. The study reported the selection of a synthetic molecule that can interact with Mdm2, and rescue p53; this molecule could be used for therapeutic purposes. Recent studies have suggested that the p53 response is differentially regulated in normal and cancer cells,\(^ {53,54}\) and have reinforced the idea that p53 stabilizing molecules can be specifically used in controlling cancer cells. An increasing understanding of the biochemistry of the p53 pathway is allowing us to develop novel therapeutic strategies by manipulating this pathway. This illustrates how basic research is being applied to clinical applications in cancer.\(^ {55}\) Indeed, the first commercial p53-based production of gene therapy drug named Gendicine has been approved recently in China. The Food and Drug Administration of China has licensed Shenzhen SiBono GenTech to produce and market recombinant Ad-p53 gene therapy for head and neck squamous cell carcinoma.\(^ {56}\)
Azurin from Pseudomonas aeruginosa can physically interact with p53. It preferentially binds to the N-terminal and middle region of the p53 but only weakly to the C-terminal. To the best of our knowledge azurin is the first bacterial protein reported to be interacting with p53, and hence it has the potential of being used in p53-based anti-cancer therapies. It modulates p53 activity and induces the apoptotic cell death (Fig. 1). Interestingly intraperitoneal injections of 0.5 mg of azurin to a group of immunodeficient nude mice harboring xenografted Mel 2 tumors allowed tumor regression to the extent of 59% in 22 days. Multivariate analysis based on a random coefficient model showed that the difference in tumor volume between treated and control animals was statistically significant. Moreover, when two hydrophobic amino acids at the 44 and 64 positions in azurin were replaced with more polar amino acids to generate a M44KM64E double mutant, it was found to have reduced cytotoxicity towards Mel 2 cells as compared to wild type azurin. This mutant triggered the inhibition of cell cycle progression in mammalian cells but little apoptosis. The oligomerization domain of p53 is located in the central to C-terminal region of p53; it might therefore be possible that the M44KM64E mutant azurin binds to p53 in this region and somehow directs the specificity of p53 for p21. p53 is a very well characterized molecule. The use of synthetic nonoverlapping p53 peptides of known function may help in characterization of their interaction with azurin/ or mutant azurin. Alternatively, gene array studies can be used as a tool to identify mammalian cell cycle genes, which are differentially regulated in cancer cells treated with azurin.

More recently, the entry of azurin into the human breast cancer cell line MCF-7 followed by induction of apoptosis has been reported. MCF-7 cells containing wild type p53 were highly susceptible to azurin treatment compared to the p53 null cell lines. The intracellular p53 level increased after azurin treatment in MCF 7 cells; however a fraction of p53 as well as a small amount of azurin was also translocated to the mitochondria. Mitochondrial p53 is associated with nontranscriptional activity to modulate cell functions. Hence further studies are needed to study the role if any of mitochondrial p53 in cell death associated with azurin treatment and analyze the relative involvement of transcriptional and nontranscriptional p53 activity. Current studies are directed at analyzing the relative importance of transcriptional and nontranscriptional p53-dependent cell death in response to azurin treatment. A clinically important finding of Punj et al. was that azurin allowed significant regression of breast cancer in vivo in athymic mice injected with 1 mg of azurin daily as compared to untreated mice. A mean tumor volume of 15% was observed 29 days after the start of the experiment in treated mice as compared to controls, demonstrating 85% regression of tumor growth. There was no apparent loss of body weight of mice or any other visual side effects of azurin treatment. The immuno-histological examinations of tumors showed apoptotic cell death in tumors, with a marked increase of disintegrated parenchyma and reduction in proliferating cells in azurin-treated mice over untreated control.

One of the major concerns in using foreign proteins as therapeutic agents is their associated immunogenic and inflammatory response. Immune responses to therapeutic proteins can affect therapy by inducing inflammation and unwanted immune responses such as autoimmunity. The mitogenic effect of some bacterial proteins can also induce the polyclonal activation of T and B cells with possible clinical complications. In view of this, we tested if azurin can induce an immune response in C57BL/6 mice treated with 1 mg/ml of azurin, intra-peritoneally daily for 28 days, as reported in previous studies. The mice were monitored for 40 days, bled every 10 days, and tested for azurin specific antibody response. After 40 days, mice treated with azurin demonstrated a very low level of azurin-specific antibody (titer of 1:800), predominantly of the IgG class, which is insignificant considering that these mice had received repeated injections of azurin. Spleen cells from these mice showed no significant proliferative response as determined by $^3$H-thymidine incorporation assay. There was no change in the number of T cell sub-populations (CD4$^+$ and CD8$^-$), B cells (CD19$^+$) and T cell activation markers (CD62L) as determined by FACS analysis, suggesting that azurin has no significant antigenic or mitogenic activity.

It is interesting to note that Punj et al. found that azurin has less apoptotic effect in nontransformed cells such as MCF10F, HBL 100 or foreskin cells as compared to MCF-7 cells, suggesting that perhaps cancer cells are preferential targets for azurin treatment. It is still not known how azurin enters into cancer cells and if any receptors are involved in azurin trafficking. Studies on the mechanism of entry of azurin into mammalian cells might explain the affinity of azurin for transformed cells. Certain antibacterial peptides have been found to have impressive in vitro and in vivo activity against cancer cells without damaging the normal eukaryotic cells. Expression of antimicrobial peptides such as cecropin B1 (CB1) and melittin was found to produce anti-tumor properties. CB1 is a derivative of a...
natural antibacterial peptide Cecropin B, and has a higher specific activity against cancer cells than its natural form Cecropin A (CA). This specific activity has been attributed to unique structure of CB1 consisting of two-ampipathic helical segments. However the mechanism by which peptides kill both prokaryotic and transformed eukaryotic cells is not well understood. It is believed that the extent of secondary structure as they approach the cell membrane, followed by the contents of cationic residues, may play an important role in the lysis of cell membranes. However it is not clear whether it is the protein structure or the sequence which plays a predominant role in the lysis of the cell membrane.

An ideal anti-cancer agent will be as small a molecule as possible with low levels of side effects. Azurin induces a low level of cell death in normal cells. It might be possible that only a portion of the azurin molecule may be required for interaction with p53 leading to induction of cell death. The His-tag fused truncated azurin proteins were purified using Ni-NTA spin columns, and used for His-pull-down assays to study the interaction with p53. Azurin fragments encompassing amino acids 30–94 and 71–150 (corresponding to the predicted amino acid sequence) showed binding with full-length p53 (Fig. 2). It is interesting to note that azurin fragment 30–94 is an amphipathic peptide with both hydrophobic and hydrophilic domains. Such peptide could be targeted with a tumor specific antigen and used in combinational bio-chemotherapy in order to optimize the dose. One of the important example of biochemotherapy is the use of Dacarbazine (DTIC) and Interferon-α (IFN-α), which is an FDA approved and is widely used therapy in metastatic melanoma. The clinical significance of this azurin fragment should be investigated in order to develop a peptide-based anti-cancer agent.

The question which arises, is why bacteria would secrete such a redox protein to kill mammalian cells? It has recently been proposed that it is the physical presence of redox enzymes rather than their enzymatic activities, in the cytosol of the mammalian cells, which sends a signal to the cell of an impending energy catastrophe. Generally, redox proteins such as AIF (apoptosis inducing factor) and cytochrome c are present in the mitochondrial inter-membrane and not in the cytosol. Mitochondria, which are believed to have prokaryotic ancestry from protobacteria, are the energy storehouse of the mammalian cells. In response to death stimuli, mitochondrial redox proteins are released into the cytoplasm and trigger cell death. It is interesting to note that ancestors of protobacteria, the mitochondria, and the present day bacteria such as P aeruginosa retain the evolutionary conserved function of releasing redox proteins to trigger apoptosis in mammalian cells.

CONCLUSION AND PERSPECTIVES

Bacterial products such as epothilones, lipotechoic acid, peptido-glycans, toxins and especially, the redox proteins of prokaryotes such as Pseudomonas aeruginosa in particular can modulate the mammalian cell functions. These products lead to cell death, by entering the host cells either in p53-dependent or independent mechanisms. Since azurin showed in vivo regression of tumor growth without inducing any significant toxic side effects, this promising anti-cancer agent should be further studied in order to understand its translational significance. Further understanding, the mechanism of action of azurin and its p53 interaction will provide us with the basis to further improve the potency of this agent and optimize its dose. It should be possible to preferentially develop a short azurin-based synthetic peptide, which could be used in the fight against cancer. Further in vivo studies are in progress to determine if azurin can be used as an alternative means of gene therapy in solid tumors.

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