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Bacterial and Liposomal Vector Guided Drug Delivery System via Tumor Markers Carrier Gene to Treat Neoplasm

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Abstract

The main goal of cancer therapy is to not only treat cancer but also to protect healthy cells from toxic effect of chemotherapeutic agent. For this purpose, different modern techniques are devised. Significant localized chemotherapeutic effect can be induced by combining all these techniques. Bacteria have been used as a novel organism for chemotherapy. Many new chemotherapeutic drug delivery techniques have come into existence these techniques involve bacterial cloning. The cloned bacteria contain either drug carrying gene or a prodrug activating enzyme. Bacteria chosen for therapy should be able to grow in hypoxic tumor condition. Experiments done on clostridium (anaerobic bacterium), produces tumor suppression in many animals.

Keywords: Chemotherapy; Bacteria; Doxorubicin; Clostridium; Tumor

Introduction

In the field of cancer chemotherapy, a systemic way of treatment is required to cure both types of tumors, primary and metastatic tumors. This approach needs some sort of agent and factors for targeting the tumors. For this purpose, bacteria, bacterial derived drugs, toxic agents, bacterial vectors are studied. Most commonly used bacterial vector for delivering foreign DNA into the tumor cells are *Salmonella typhi*, *Bifido bacterium*, *Salmonella choleraesuis*, *Vibrio cholera*, *Listeria monocytogenes* and *Escherichia coli*. For example DNA from attenuated strains of Salmonella has been used to suppress melanoma [1-13]. Many bacteria like Salmonella, Clostridium and *Escherichia coli* have the properties of adhesion to tumors i.e., they can colonize in tumor cells. Scientist utilizes these adhesive properties of bacteria in order to treat various tumors[14] .Genetically modified tumor adherent bacteria can be used for anticancer therapy by utilizing approaches like bactofection, DNA vaccination, delivery of bacterially mediated protein and RNA [13]. Vaccines derived from Cooley work has led to the development of potent anticancer drug (Doxorubicin) from *Streptomyces peucetius* [3]. Studies suggested that clostridium species from *C. novyi* NT enhances the release of liposome encapsulated drugs which is used on *COBALT* therapy for treatment of cancer [3]. Pro-biotic bacteria like Lactobacilli also found to adhere with tumors and can be used for treating cancer. The significance of bacteria in cancer therapy was acknowledged almost a century ago. A German physician W. Busch and F. Fehleisen worked individually and observed that certain types of cancers reverted in the patients having erysipelas infections accidently. An American physician, William Coley, observed that one of his patients having neck cancer started to recover in the presence of erysipelas infection. He was the first who properly documented the usefulness of bacteria and bacterial toxins for treating cancers even at last stage. He constructed a vaccine by using two bacterial species, *Streptococcus pyogenes* and *Serratia marcescens* in killed form to produce an infection along with fever with no risk of actual infection. This vaccine was used to cure sarcomas, lymphomas, carcinomas and melanomas successfully. In many cases of advanced malignancy complete and long term regression was also reported. Bacterial derived toxins 'Coley toxins' were also examined for their anticancer activity. This brilliant discovery of Coley's toxins was proved a milestone for the advance research in this field [3]. Many anti-cancer drugs are also naturally derived e.g., Bleomycin, Lidamycin Doxorubicin and Dactinomycin [15]. Gene encoding these drugs can be manipulated into anaerobic bacterium so that they exert their pharmacological effect in cancerous cell only.

Cancer and Chemotherapy

Cancer is a disease characterized by uncontrolled growth of cell in any area of the body. There are two types of cancer i.e., benign and malignant. Studies suggested that, to have cancer in genome, a person should have 5 to 6 mutation in his genome. For benign cancer at least 1 additional mutation must occur [16]. Tumor cell grows very rapidly and form a cellular mass. This cellular mass deprived internal cell from getting proper oxygen hence histological examination of cancer cell shows that the area of necrotic cells in the interior [17]. This hypoxic environment hinders the penetration of chemotherapeutic drugs inside tumor. Hence, conventional chemotherapy cannot be a wise treatment strategy. Recent trends in chemotherapy utilize bacterial mediated enzyme chemotherapy. In this therapy, various aerobic and anaerobic bacteria have been utilized in order to deliver drug to tumor cells.

Prodrugs and Its Use in Chemotherapy

Pro-drugs are the drugs that are inactive when administered but become activated when react with specific enzyme [18]. In chemotherapy pro-drugs are used in order to increase drug penetration in tumor cells [18]. If drug is activated outside the tumor, anticancer drug also destroy healthy cells along with tumor cells by bystander effect [18].

Major vector employed in order to activate prodrugs

Pro-drug needs specific enzyme(s) in order to be activated and perform its action. To enable pro-drug to perform its action, enzyme that activate pro-drug should be present in bacteria. Furthermore

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bacteria should secrete enzyme and activate drug in tumor cells only. The vectors required for bacterial directed enzyme pro-drug therapy are as follows.

Vectors employed for activation of pro-drugs are:

Bacteria: Most commonly employed for BDEPT are Clostridium, Salmonella**,** Bifidobacterium and **Escherichia coli** [18].

Virus: Generally viruses are not employed as vector because they have short genome [18]. Most commonly employed viral vectors are Adeno-associated virus (AAV), Adenovirus (AdV), Retrovirus and Herpes Simplex Virus (HSV) [18].

Natural proteins: Most commonly employed are antibody [18].

Synthetic vectors: Most commonly employed are liposome [18-26].

Major enzyme employed for activation of prodrugs

Pro-drugs inside the tumor must be activated in order to exert its tumor suppressing effect [18]. Cleavage of pro-drug by an enzyme should release a labile drug that can able to suppress tumor [18]. Elevated level of certain enzyme can be used as indicator of various types of cancer such as elevated level of cathepsin D with pro-form is the indicator of malignant disease progression in breast cancer [18-36]. Pro-drugs with doxorubicin (DOX), Mitomycin C (MMC) and Paclitaxel (taxol) are mostly attached to cathepsin B-sensitive di-peptide [18]. Pro-drugs containing glucuronide moieties are activated by β-Glucuronidase [18]. Human prostate contain high level of acid phosphatase which can be used to activate pro-drugs containing acid phosphate [18-45].

Strategy for delivery of chemo therapeutic drug inside tumor

Pro-drugs are usually employed for chemo therapy because they are less toxic and have more penetrating power for tumors [46]. For this purpose to activate a cytotoxic pro-drug, following strategies are employed [46].

a. Pro-drug should be readily transported to the site where tumor is present [46].

b. On reaching tumor site, it should be activated by enzyme, secreted by bacteria or by enzymes expressed by tumors [46].

c. Active drug must exert its action on tumor tissue by retaining in tumor tissue [46].

Utilizing Characteristics of Tumor to Target Prodrug/ Drug

Hypoxia

Physiology of tumor tissue is different from normal tissue. Normal tissue have ordered blood vessel but tumor have leaky capillaries. These differences inhibit drug from penetrating drug inside the tumor [45-55]. Hypoxia is the main characteristics of solid tumors. Hypoxic environment activates reductase enzyme [1]. So we can employ an anticancer pro-drug that can activated when reductase act on it [1]. Common pro-drug activating enzyme employed for this purpose is nitroreductase. This enzyme can work on hypoxic environment. Drug activated by this enzyme is called nitrogen mustard, having anticancer activity against lymphomas [1,56-62]. Clostridium strains cloned for producing enzyme nitroreductase are employed that convert pro drug CB1954 to nitrogen mustard (a cytotoxic agent) [5]. Studies prove that many bacteria can colonize effectively in tumor tissue. For this purpose, gene of cytosine deaminase from *E. coli* is cloned to *C. sporogenes* to activate prodrugs like 5-FU [63]. This therapy produces greater therapeutic effect than conventional therapies [21].

Receptors and protein expressed by tumor

For the purpose of localized chemotherapy, it is necessary to identify tumor related antigens so that selectivity can be improved. That's why antitumor drugs are coupled with antibodies so that they could attack these antigens. This process improves tumor selectivity [22]. Angiogenesis tumor blood vessels express some tumor markers that make tumor cells different from normal cells, many of these marker proteins are also involved in angiogenesis [23]. Targeting these marker proteins can inhibit malignancy. One of these marker proteins is folate receptor expressed by cancer cells of breast. These receptors decrease the uptake of antitumor drugs to the tumor cells. Identifying this receptor and using liposome vector are major approaches to cancer therapy. Folate entrapped copolymer of Poly Ethylene liposomes are used in order to deliver anticancer drugs to specified tumor [24]. Major anticancer drugs which are used with folate liposome are Doxorubicin (an anticancer antibiotic) [24]. Studies suggested that mice suffering from oral carcinoma express folate receptors which when treated with Doxorubicin - folate liposome causes reduction in tumor size [25]. Another protein called EPH protein also expressed in tumor cells its concentration increase in angiogenesis [34]. By targeting these proteins through bacteria angiogenesis can be prevented. During metastasis of breast cancer, CXCR4 and CCR7 receptors are highly expressed, there are involved in metastasis of breast cancer by blocking these receptor through antibodies, metastasis of breast cancer can be avoided [35].

Anaerobic Bacteria as Major Vector for Anticancer Drug Delivery

Cytotoxic activity can also be induced directly to tumors cells by incorporating gene with anticancer activity to anaerobic bacteria [3]. In this context, various anaerobic bacteria have been identified and used most commonly employed bacterium is clostridium as m-55 [3]. Other strains of bacteria that are in research are *S. typhi*, bifido bacterium *Salmonella choleraesuis, Vibrio cholerae, Listeria monocytogenes* and *Escherichia coli* respectively [4]. Tumors typically show anaerobic environment so employing anaerobic bacteria to tumor result in localized cytotoxic response [3]. Studies shows that incorporating doxorubicin gene obtained from *Streptomyces peucetius* in clostridium, does not result in much tumor regression .In contrast *S. typhi* shows significant tumor regression but, it can grow in aerobic as well as anaerobic environment so it can cause damage to healthy cells [4]. By incorporating hypoxia inducible promoter to *S. typhi*, specificity of drug delivery can be increased [5]. Anaerobic bacteria take advantage of hypoxic tumor environment in which there is less blood supply with tissue necrosis [6]. Necrotic tumor environment provide purines that helps to grow anaerobic bacteria in necrotic and tumourogenic environment [6]. Necrotic tissue also contains chemo attractant compounds like aspartate, serine, citrate, ribose or galactose which help bacteria to reach to the place where tumor is present [6].

Delivery of doxurubin gene to anaerobic bacteria

Doxorubicin is an anticancer antibiotic drug that is obtained from *Streptomyces peucetius* [2]. It is an anthracycline antibiotic, which produces its anticancer effect by inculcating into DNA [7]. It is drug of choice for acute leukemia, lymphomas, soft-tissue and estrogenic sarcomas, pediatric malignancies and adult solid tumors' in case of breast and lung carcinomas [7]. As it is obtained from bacteria so by manipulating its gene in anaerobic bacteria it can be utilized for bacterial directed localized chemotherapy. Most common strain of

Streptomyces peucetius that produces high yield of antibiotic is 29050 [8]. By isolating *doxorubicin* gene from *Streptomyces peucetius* an incorporating in anaerobic bacteria like clostridium or *E. coli* can be a remedy for effective chemo therapy. Scientist found that pathogenic specie of bacteria *C. novyi* grows extensively in necrotic tumor and produces significant tumor regression as a result of that observation, many new method of localized chemotherapy can be devised [9]. Following table shows the presence of drug carrying gene with its therapeutic use (Table 1).

Role of Bacteria in Prodrug Activation

Bacteria play a very important role in pro-drug activation. Bacteria naturally produce certain enzymes that can activate pro drugs. Furthermore, environment expressed by tumor is different from healthy cells. This difference in environment is readily sensed by bacteria [36]. After administration of bacteria in tumor, bacteria sense tumor environment and selectively replicate in it [36]. During replication bacteria produce certain enzymes that can be used to activate nontoxic pro-drug [36]. These bacteria have axotropic mutations which make these bacteria highly safe for antitumor therapy. Trials for these drugs, based on bacteria are done on mice which show delay in tumor growth when recombinant bacteria activation pro-drug is administered [36]. Various drugs are activated by bacterial enzyme and these strategies can work for localized chemo therapy. Bacterial enzyme namely alkaline phosphatase, carboxypeptidases, beta-glucosidases and beta-lactamases are being utilized for activation of cytotoxic pro-drugs [47] (Table 2).

Lipid Based Liposome for Chemotherapy

Incorporating, chemotherapeutic drug in lipid based nanoparticles (liposome) can be used for localized chemotherapeutic drug delivery [48]. For this type of localized delivery, specific tumor markers are incorporated in these nanoparticles along with chemotherapeutic drug [48]. Markers attach with nano particles attach the liposome system drug to tumor site and after attachment drug is released [48]. Doxurubin was the first anticancer drug which was incorporated in liposome particles for delivery into tumor cells [48]. Thermo sensitive liposomic particles are also created [48]. These particles release drug when they feel temperature difference [48]. The temperature of tumor site is raised as compare to other body these characteristics can be utilized for further treatment options [48]. polyethylene glycol is the agent that can in corporate intact cells in its structure so , bacteria carrying drug gene can be incorporated in liposome so that it could help bacteria to be taken from site of action and release there [49].

Conclusion

Future approach for cancer treatment would not only to destroy cancerous cells but also to save healthy cells. For this purpose, both synthetic and bacterial vectors will be employed. Bacterial vector should contain drug carrying gene or prodrug activating enzyme and liposome contain tumor markers. Combination of bacterial and liposomal vector system will be tool for localized chemo therapy. For example, in case of breast cancer, folate receptors are over expressed. So by incorporating genetic engineered clostridium in folate liposome can be tool for localized chemotherapy. Studies prove that cancerous cells uptake more frequently folate contains liposome than non-liposomal system [50].

Significance of choosing bacterial vector

By choosing a suitable bacterial vector, and cloning a suitable gene to it, we can deliver chemotherapeutic drug or prodrug activating enzyme to it. As anaerobic bacteria grow efficiently in hypoxic environment and tumor environment shows hypoxic conditions. So growing of anaerobic bacteria in tumor become easy and it can also contribute to localized chemotherapy

Significance of using combination techniques

Using combination of vectors for the delivery of chemotherapeutic drug is convenient way of chemotherapy, as in breast cancer folate receptors express in massive way. Using folic acid liposome internally incorporated with genetically modified clostridium with doxurubin gene is acceptable treatment strategy for localized chemotherapy. So, the overall local chemotherapeutic response is produced by combination of the techniques may save healthy cells and in turn destroy cancerous cells.

SR.NO	NAME OF ORGANISM	DRUG PRODUCED	MECHANISM OF ACTION	USE	JOURNAL NAME
1	*Streptomyces verticillus* [27]	Bleomycin [26]	Mediate oxidative degradation of all cellular RNAs [53]. It bind to selective DNA sequence, which is GC rich and destroy DNA [53,54]. Act in G2 phase of cell cycle[54]	Hodgkin's lymphoma, squamous cell carcinomas, testicular cancer, plantar warts [27]	Journal of Dermatology [27]
2	*Streptomyces parvullus* [28],	Dactinomycin [26]	It inhibit DNA dependent RNA synthesis, by binding to that portion of DNA that is going transcription [55-57]	testicular cancer and Ewing's sarcoma [28]	Hand book of oncology [28]
3	*Streptomyces caespitosus* or *Streptomyces lavendulae* [29]	Mitomycin [26]	It bind with thioredoxin reductase enzyme ,present normally in tumors and in activate its active site that contributes its cytotoxicity [58]	anal cancers, and breast cancers [29]	J Molec Biol [29]
4	*Streptomyces galilaeus* [30]	Anthracyline [26]	Inhibition of topoisomerse 11 enzyme by Intercalate between adjustant DNA base pairs [59] Produce hydoroxyl free radicals [59]	Solid tumors and hematological malignancies [30]	American chemical society [30]

6	*Streptomyces peucetius* [31]	daunomycin and doxorubicin (adriamycin) [26]	It intercalate into DNA by inhibiting topoisimerase11 enzyme [60] It produces free radical that damages DNA , RNA and Proteins [60]	Breast cancer [31]	Seminar in oncology [31]
7	*Micromonospora chersina* [32]	Dynemicin A [26]	It binds with DNA strand and cleaves it. preferred cleavage site for dyenomicin is 3' side of purine bases [61]	leukemia, breast and lung cancers [32]	Chemistry groups [32]
8	*S. globisporus* C-1027 [33]	Lidamycin [26]	It damages DNA by radicle mediated hydrogen removal	Myeloma [33]	Beijing Union Medical College [33]

Table 1: Table shows various organisms that contain gene for anticancer drug with use.

SR.NO	NAME OF BACTERIAL STRAIN	GENE EXPRESSED	ENZYME	DRUG PRODRUG	MECHANISM OF ACTION	TYPE OF CANCER CURED	JOURNAL NAME
1	Salmonella [37]	HSV TK [37]	thymidine kinase polypeptide [37]	phosphorylation of the prodrug ganciclovir [37]	It inhibits deoxyguanosine triphosphate (dGTP) incorporation into DNA [62]	Melanoma [37]	The American Journal Of Cancer(1931-1940) [37]
2.	*Escherichia coli* [38]	--------	uridine phosphorylase [38]	Capecitabine (Xeloda, N4-pentyloxycarbonyl-5'deoxy-5-fluorocytidine)to anticancer agent 5-FU [38]	It inhibit thymidylate sybthetazae enzyme [63] It also block conversion of 5, 10-methylene tetrahydrofolate to dihydrofolate [63] It also inhibit conversion of dUMP to dTMP which is necessary for DNAsynthesis [63]	Colon, rectum, and head and neck cancers. [38]	Curr med chem [38]
3	Mainly produced in rabbits which can be cloned in bacterium [39]	----------	Carboxylesterase [39]	TAX prodrugs, 2'-ethylcarbonate-linked paclitaxel [39]	Bind with microtubules and stabilizes microtubule, thus block G2/M phase of cell cycle. [64]	Ovarian cancer [39]	-------
4.	*E. coli* [40]	----------	Nitroreductase [40]	Pro-drug CB 1954 is activated to the cytotoxic metabolite 5-aziridin-1-yl-4-hydroxylamino-2-nitrobenzamide [40]	Highly toxic DNA cross linking agent [65]	Lymphomas [1]	Pharmacological reviews [40]
5.	*Oceanobacillus iheyensis* [51]	----------	methionine synthase reductase (MTRR), aldo-keto reductase 1C3 (AKR1C3 [41]	CONVERSION OF PR-104 TO PR-104A [41]		HEPATOCELLULAR CARCINOMA [41]	Auckland Cancer Society Research Centre [41]
6.	*Escherichia coli* and *Mycobacterium tuberculosis* [42]	--------	Nitric oxide synthases (NOSs) [42]	5-(Aziridin-1-yl)-2,4-dinitrobenzamide (CB1954) [42]	Highly toxic DNA cross linking agent [65]	Human ovarian cancer [19]	Proc Natl Acad Sci U S [42]

7.	Mainly prodced in rabbits which can be cloned in bacterium [18]	----------	carboxyesterases enzyme [18]	Convert Irinotecan (CPT11) to camptothecin (SN38) [18]		Adeno carcinoma and squamous cell carcinoma [20]	Recent trends in antcancer therapy [18]
8.	Present before development of malignancy in cancers [52]	-------	Matrixmetalloproteinases-2 [43]	CBI-TMI (a duocarmycin class minor groove binder) [43]		Brest cancer [43]	Molecular pharmaceutics [43]
9.	*Salmonella typhimurium* strain SL7838-chrR6 [12]	------	ChrR6 [44]	6-chloro-9-nitro-5-oxo-5H-benzo(a) phenoxazine (CNOB). [44]		Treat various tumor in mice [44]	Molecular cancer ther [44]

Table 2: Table showing prodrugs along with activating enzyme.

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