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Antibody Conjugated Polymeric Prodrugs the Future for Cancer Therapy

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Abstract Cancer is one of the most devastating conditions affecting humans. Current drugs used in the treatment of cancers and other diseases are becoming ineffective as a result of drug resistance, occurring naturally as well as the abuse and misuse of drugs as well as issues of patient compliance to therapy due to its high toxicity. To overcome this resistance and improve the efficacy of these drugs, a selective drug delivery is essential. Such selective or targeted delivery is the use of antibody conjugated polymeric drugs in the treatment of cancers. A targeted delivery of cytotoxic agents to tumors is believed to improve both their anti-tumor effect and safety. This review focuses on the recent cancer drug therapy such as chemotherapy, immunotherapy, hormone therapy and antibody drug conjugates as well as research on new therapies such as polymer drug conjugates and antibody conjugated polymeric prodrugs. Challenges encountered in antibody conjugated polymeric prodrugs for cancer treatment and measures to improve it such as nanosizing the delivery systems are also discussed.

Keywords *Cancer; Cancer Therapy; Drug Delivery; Targeted Therapy; Antibody Conjugated Polymeric Prodrugs; Nanoparticles*

1. Introduction

Current therapies for treating diseases are gradually failing. Once life saving antimicrobials, parasitic and antineoplastic drugs is losing their potency and in no time will be ineffective due to drug resistance. Disease causing organisms and pathologies has not only become drug resistance but multidrug resistance where the organisms become resistance to more than one drug. It is also known that drug resistance traits are passed on to offspring which will result in a population that is more resistant. The primary questions are why are these drugs failing us? Why the resistance? Will drugs currently being used also be ineffective in the near future? What then is the future for us?

Drug resistance can be attributed to a number of factors. A number of central genes and pathways have been known to be associated with drug resistance. For instance in cancer therapy, resistance can be produced either by intrinsic causes (such as by mutant genes) or by external causes (such as

by signaling from the microenvironment), it can develop as a single step or as multiple steps of random genetic mutations or any other abnormality occurring in gene products which can be the consequence of drug administration, drug properties, cell location or can be acquired independently of any drug [1]. Heterogeneity within tumor cells as well as over expression of the ATP-binding cassette transporters and multidrug resistance-associated proteins and breast cancer resistance proteins have all been complicated in chemotherapy drug resistance [2]. The above mentioned proteins bind to a substrate such as a drug in the membrane and subsequently expel it into the extracellular environment leading to sub therapeutic intracellular drug concentrations and subsequently rendering tumors refractory to chemotherapy due to the demand for higher toxicity-limiting drug doses [3].

Conventional therapies which lacks tissue specificity and results in a higher dose of pharmacological agent within the body resulting in systemic toxicities [4] is also believed to be a contributing factor to failed therapy as patient compliance to therapy is hindered. It is therefore realized drug resistance develops naturally, but careless practices in drug supply and use are also hastening it unnecessarily. There is no doubt modern conventional therapies will fail us in decades to come. It will therefore be essential to find other alternatives which will escape these drug resistance challenges and drug ineffectiveness.

To combat drug resistance and challenges associated with conventional therapy as well as improve the efficacy of current therapies, the need arises to develop novel biomedical technologies that will require the efforts of clinicians, biomaterial scientists as well as biomedical engineers. Such technologies include polymeric drug delivery systems and nanomedicine-based therapeutics. This review focuses on the current therapies in place for the treatment of cancer and examines recent research of improved therapies to overcome challenges in these current drug therapies. It also discusses some challenges associated with these improved therapies and how to overcome these challenges to develop an efficient macromolecular drug carrier system for cancer therapy.

2. Cancer and Current Drug Therapies

Cancer known medically as malignant neoplasm is one of the most devastating diseases and it involves various genetic alterations and cellular abnormalities [5]. Cancer still remains one of the world's most devastating diseases; with more than 10 million new cases every year [6]. The World Health Organization estimates that 84 million people will die of cancer between 2005 and 2015 [4]. Cancer is basically a condition which involves unregulated cell growth in which cells divide uncontrollably forming malignant tumors which may spread to other parts of the body through the bloodstream or the lymphatic system. Normal cells in the body follow an orderly path of growth, division, and death. When the process of programmed cell death lais, apoptosis breaks down, cancer begins to form. This leads to a mass of abnormal cells that grows out of control. Cancer harms the body when these cells divide uncontrollably to form lumps or masses of tissue (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). Tumors can grow and interfere with the digestive, nervous, and circulatory systems and they can release hormones that alter body function. Tumors that stay in one spot and demonstrate limited growth are generally considered to be benign. Malignant tumors are termed cancerous and are more agile than non-malignant ones.

Although the mechanisms of formation and spreading of cancer are still not well understood, both external factors (tobacco smoking, chemicals, radiation, and infections) and internal factors (inherited metabolism mutations, hormones, and immune conditions) are believed to be relevant [7]. A person is susceptible to cancers if there are damages or mutations to DNA which damages the genes involved in cell division. There are four main types of gene responsible for the cell division process: oncogenes tell cells when to divide, tumor suppressor genes tell cells when not to divide, suicide genes control apoptosis and tell the cell to kill itself if something goes wrong, and DNA-repair genes instruct a cell to

repair damaged DNA. Cancer hence occurs when a cell's gene mutations make the cell unable to correct DNA damage and unable to commit suicide. It may also occur as a result of mutations that inhibit oncogene and tumor suppressor gene function, leading to uncontrollable cell growth. Recent results of cancer genomics have revealed that most human solid tumors are not only single gene-based events but also multiple genomic alterations [8].

Carcinogens, a class of substances directly responsible for damaging DNA also predispose a person to cancer. Tobacco, asbestos, arsenic, radiation such as gamma and x-rays, the sun and compounds in car exhaust fumes are all examples of carcinogens. When our bodies are exposed to carcinogens, free radicals are formed that try to steal electrons from other molecules in the body. Theses free radicals damage cells and affect their ability to function normally.

Cancer may also be the result of a genetic predisposition that is inherited from family members. It is possible to be born with certain genetic mutations or a fault in a gene that makes one statistically more likely to develop cancer later in life.

Aging increases the possibility of cancer-causing mutations in our DNA. This makes age an important risk factor for cancer. Several viruses have also been linked to cancer such as: human papillomavirus which causes cervical cancer, hepatitis B and C which causes liver cancer and Human immunodeficiency virus (HIV) which causes Kaposi sarcoma. A factor that suppresses or weakens the immune system and inhibits the body's ability to fight infections also increases the chance of developing cancer.

Cancer can be treated by surgery, radiation therapy, gene therapy, immunotherapy, chemotherapy or monoclonal antibody therapy depending on the location and grade of the tumor as well as the state of the disease and the condition of the patient. Surgery is the best known treatment for cancer which has not metastasized by completely removing the cancer from the body. Radiotherapy destroys cancer by focusing high-energy rays (gamma rays or x-rays) on the cancer cells which causes damage to the molecules that make up the cancer cells and leads them to commit suicide.

Chemotherapy agents are cytotoxic drugs used to treat cancer that function by targeting fast growing cells and by blocking some critical elements of the cell division process, impairing mitosis as well as promoting apoptosis [4]. Chemical drugs are generally considered to be one of the most efficient forms of cancer therapy [9, 10]. Chemotherapy utilizes chemicals that interfere with the cell division process, damaging proteins or DNA so that cancer cells are self destroyed. These treatments target any rapidly dividing cells (not necessarily just cancer cells), but normal cells usually can recover from any chemical-induced damage while cancer cells cannot. Chemotherapy is generally used to treat cancer that has spread or metastasized because the medicines circulate throughout the entire body. Chemotherapy treatment occurs in cycles so the body has time to heal between doses. However, there are still common side effects such as hair loss, nausea, fatigue, and vomiting. Combination therapies often include multiple types of chemotherapy or chemotherapy combined with other treatment options. At present, combinations of different chemotherapeutic drugs in a chemotherapy regime are an attractive strategy for effective anticancer treatment [5]. Different types of synthetic and natural anticancer drugs have been used for chemotherapy [11]. Drugs for chemotherapy include mertansine, paclitaxel, docetaxel, cisplatin, gemcitabine and fluorouracil.

Immunotherapy aims to get the body's immune system to fight the tumor. Local immunotherapy injects a treatment into an affected area, for example, to cause inflammation that causes a tumor to shrink. Systemic immunotherapy treats the whole body by administering an agent such as the protein interferon alpha that can shrink tumors [12, 13]. Immunotherapy can also be considered non-specific if it improves cancer-fighting abilities by stimulating the entire immune system, and it can be considered targeted if the treatment specifically tells the immune system to destroy cancer cells.

These therapies are relatively young, but researchers have had success with treatments that introduce antibodies to the body that inhibit the growth of breast cancer cells [12].

Several cancers have been linked to some types of hormones, most notably breast and prostate cancer [14, 15]. Hormone therapy is designed to alter hormone production in the body so that cancer cells stop growing or are killed completely [15]. Breast cancer hormone therapies often focus on reducing estrogen levels (a common drug for this is tamoxifen) [14, 15] and prostate cancer hormone therapies often focus on reducing testosterone levels [15]. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial. Contemporary methods for generating an immune response against tumours include intravesical BCG immunotherapy for superficial bladder cancer, and use of interferons and other cytokines to induce an immune response in renal cell carcinoma and melanoma patients.

Antibody drug conjugates have also been developed in the treatment of cancers. The basic strategy underlying antibody drug conjugate technology is to combine the target selectivity of monoclonal antibodies with the potency of cytotoxic agents, such as certain natural products and synthetic molecules, with the goal of generating therapeutic drugs that are highly efficacious but also safe [10, 16]. Improved understanding of tumor biology and advances in antibody engineering have made it possible to identify better tumor targets for antibody-based therapies and to generate less immunogenic humanized and human antibodies [17] which is then linked to a cytotoxic drug for cancer therapy. Example of such antibody drug conjugates is trastuzumab emtansine which targets HER2 antigen over expressed on metastatic breast cancer and gemtuzumab ozogamicin for leukemia [16]. Trastuzumab emtansine is composed of the humanized IgG1 anti-HER2 Antibody trastuzumab (Herceptin) with maytansinoid DM1 via a non-cleavable thioether linkage SMCC [10]. Most antibody drug conjugates rely on the release of the drug from the antibody after it is in the endosome in order for it to exert its pharmacological activity in the cytosol or nucleus [17, 18]. These antibody drug conjugates have higher efficacy with fewer side effects as compared to their non conjugated cytotoxic low molecular weight drugs due to their specific targeting.

3. Polymeric Prodrug Delivery Systems

Advances in polymer science have led to the development of several novel drug-delivery systems. The use of natural biopolymers in drug delivery is of great interest due to their desirable biocompatible, biodegradable, hydrophilic and protective properties [19]. Polymers are macromolecules having very large chains, contain a variety of functional groups, can be blended with other low- and high–molecular-weight materials, and can be tailored for any applications. Biodegradable polymers offer tremendous potential either as a drug delivery system alone or in conjunction with other polymers. Such polymers include vinyl polymers such as N-(2-hydroxypropyl) methacrylamide [20], synthetic poly (α-amino acids) such as poly (L-glutamic acid), polysaccharides such as chitosan and dextrans [21], proteins such as albumin, etc. Two or more different polymers may be combined to enhance their properties. Such co-polymers possess the cumulative favorable properties of its constituent polymers. For instance, cyclodextrin has the merit of a hydrophobic cavity which is easy to assemble with other molecules whiles chitosan has a merit of degradation slowly in organism without triggering any immune response. Therefore grafting cyclodextrin molecules into chitosan reactive sites may lead to a molecular carrier that possess the cumulative effects of inclusion, size specificity and transport properties of cyclodextrin as well as a controlled release ability of the polymeric matrix [22]. Chitosan grafted with cyclodextrin also have the ability to form complexes with a variety of other appropriate compounds [23].

The need for polymer-drug conjugates became necessary because most conventional low molecular weight anticancer drugs have an inherent character to transverse in and out of blood vessels freely and this causes a non-selective distribution in both normal and tumor cells causing undesirable side

effects disastrous to patients [3]. Cancer-selective targeting became an important goal for scientists [8] hence binding a drug to a polymer with its resultant increase in molecular weight significantly altered the biodistribution of the drug into the cells by endocytosis, achieving targeted delivery at both tissue and cellular levels [3]. Linking these drugs to a macromolecule such as a polymer (polysaccharides, proteins, poly amino acids) makes it possible for tumor accumulation [24] and confers on them some selectivity as well as enhancing the drugs therapeutic effects.

The concept of polymeric macromolecule–drug conjugates was first proposed by Ringsdorf for delivery of hydrophobic small drug molecules to their sites of action [20, 21, 25]. Since Ringsdorf proposal, polymer-drug conjugates or polymeric prodrugs as a special type of drug delivery system have attracted considerable attention due to their particular therapeutic properties, such as prolonged half-life, enhanced bioavailability, lower immunogenicity and antigenicity, and often targeting specific cells, tissues or organs [9, 26, 27, 28, 29].

3.1. Drug Conjugation to Polymer

An oversimplified model of a polymer–drug conjugate consists of a biocompatible water-soluble polymer carrier bearing in its side chains drug moieties and homing device with the carrier being either an inert or biodegradable polymer forming the backbone of the system and protecting the drug from fast elimination from the body [18] as depicted in Figure 1.

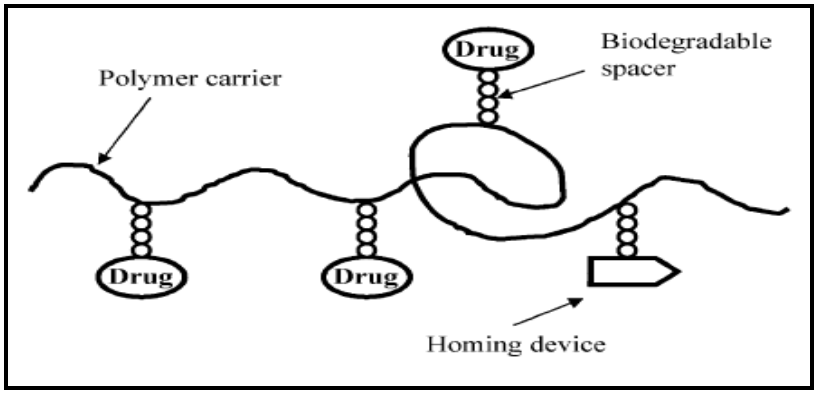

Figure 1: *Scheme of a Polymeric Drug*

A polymer-drug conjugate comprises a variety of complex macro molecular systems, their common feature being the presence of a rationally designed covalent chemical bond between a water-soluble polymeric carrier and the bioactive molecule(s) [3, 27]. The bioactive material or drug can also be encapsulated within the polymer, form non-covalent complexation or conjugate to the polymer via liable linker [20, 29]. Such linkers include oligopeptides [30] and oligosaccharides and these linkers must only degrade at the lysosomal environment [31]. The drug can be introduced to the polymeric drug delivery system by either copolymerization with a monomeric drug derivative such as N-(4-aminobenzensulfonyl)-N'-butylurea or by polymer-analogous reaction with side chains carrying functionalized groups such as active esters [3]. In the conjugation of the drug to the polymer, the chemistry of the conjugation should be able to maintain the original structure of the drug after the drug release as any change in the chemical structure of the drug may result in the loss or change of their therapeutic activity [28].

3.2. Distribution, Cell Internalization and Drug Release

To better understand the mechanisms of polymeric prodrug distribution, internalization within a cell and drug release, knowledge about the tumor microenvironment both intracellular and extracellular is

essential. Once a polymeric prodrug enters systemic circulation after inoculation, the size, shape, surface charge, and decorations, and mechanical properties of the conjugate play key roles in biodistribution, vascular dynamics, targeting, clearance, uptake, drug release kinetics and degradation [2, 3].

Polymer-drug conjugates stay longer in circulation than low molecular drugs used in chemotherapy since the endothelium of normal blood cells is typically impermeable to macromolecules [2]. It is necessary that the polymer-drug conjugate is stable during circulation in the bloodstream and the cytotoxic drug should only be released from the conjugate intracellularly or intratumorally [30].

As opposed to low molecular weight drugs which diffuse through normal vasculature to any tissue and internalize, macromolecules such as drug-polymer conjugates gain entry into tissues only through gaps in angiogenic vessels [2, 8]. This mechanism of entry by low molecular drugs accounts for its high toxicity. Polymer bound drugs enter tumor cells through fluid phase pinocytosis, dependent on the particle morphology and surface charge [2, 30]. Pinocytosis results in the internalization of solubilized material either contained in the extracellular fluid or adherent to the plasma membrane into the endosomes, which are membrane, bound vesicles coated by the protein clathrin [3]. The internalized conjugate is then carried to the early endosome which serves as a sorting compartment after which molecules obtained are recycled into the plasma membrane or the late endosome and lysosome for enzymatic degradation. Lysosomal degradation is an important step in delivery systems, the low pH of about 5 in the lysosome and lysosomal enzymes play a crucial role in this regard as this creates the optimum environment for acid-labile linkers or spacers linking the drug to the polymer in some conjugates to degrade and subsequently release the conjugated drug. A number of studies have shown that extracellular pH in tumors is consistently acidic and can reach pH values of 6.0 this being the result of the accumulation of tumor derived metabolic acids and hypoxia because of poor circulation within the tumor [2, 30]. This relatively low extracellular pH environment also plays an important role in hydrolyzing the acid-liable linker or spacer for drug release, a phenomenon known as pH controlled drug release [30, 32]. Macromolecules are retained longer and more efficiently in the tumor cells due to the EPR effect [3, 33], a major rationale for using polymeric prodrugs which is discussed later. Upon internalization into the cells, the drug from the polymer carrier can exert its therapeutic effect either from within the endosomal compartment or upon release into the cytoplasm [3].

In vitro drug release studies for analyzing polymer-drug conjugates include the dialysis method [34], high-performance liquid chromatography method [35] and the dispersion method [30]. Equipment such as a USP dissolution apparatus V from Pharmatest® Germany can also be used in vitro drug release studies [36]. In the mechanism of drug release, a too fast release can abolish the advantages of polymer conjugation and yield conjugates with the same toxicity of free drugs whereas a too slow release can impair drug activity [37, 38]. An efficient polymeric prodrug must therefore demonstrate a controlled release over a period of time.

3.3. Metabolism/Degradation of Polymers after Drug Release

Biodegradable polymers in polymer drug conjugates will undergo biodegradation either hydrolytically or enzymatically with the degradation products absorbed in the biochemical pathways. Semi-degradable polymers are degraded into smaller blocks during lysosomal degradation and excreted by the kidney. Elimination of non-degradable polymers is hampered by their high molecular weight and hence remain in the tissue after cellular death or undergo exocytosis and then via the lymphatic circulation into the bloodstream for elimination by the kidneys depending on their size [2].

3.4. Overcoming Drug Resistance

Resistance to chemotherapeutic drugs in cancers is mainly due to the active transport of these drugs out of the tumor cell mediated by certain proteins such as ATP-binding cassette transporters and multidrug resistance-associated proteins [33]. Since drug conjugated polymers changes the path of drug internalization from diffusion to endocytosis bypassing the efflux pumps (altering absorption pathway from transcellular to paracellular or transcytosis routes), it minimizes the drug interactions with multidrug resistance transporters leading to increased intra-cellular accumulation and enhanced efficacy of the drug in resistant cells as realized in a number of studies using HPMA-copolymer doxorubicin conjugates in resistant ovarian carcinoma cells [2]. Studies with Pluronic®, an inert block copolymer comprising of hydrophilic ethylene oxide and hydrophobic propylene oxide blocks, has demonstrated noteworthy improvement in the cytotoxicity of daunomycin with Pluronic® as compared to free drug in multidrug resistant cell lines [3].

Polymeric drugs may also elicit the “bystander effect” a phenomenon whereby non targeted neighboring tumor cells also perish due to the diffusion or gap junction transfer of the active drug after its dissociation from the carrier within the targeted cells [2]. This phenomenon leads to a high therapeutic effect which plays a crucial role in drug resistance elimination.

In addition polymer-drug conjugates have other advantages such as:

a) They require less administration due to increased tissue and blood half life which is of great importance to patients, allowing for patience compliance and quality of life as well as cost effective [8]. Example is in the treatment of patients with hepatitis C and cirrhosis, the use of native interferon-α which requires more than 2 to 3 injections per week whiles pegylated interferon-α (Pegasys®) requires once a week injection [39].

b) They enhance a drugs solubility, cellular uptake, intestinal absorption and physical stability. For instance, Taxol® and caplitaxel are converted to highly soluble drugs [8].

So far several polymer drug conjugates have been approved for clinical application or clinical trials. Poly (styrene-co-maleic acid)-neocarzinostatin is used for the treatment of liver cancer in Japan [28, 30] and PGA-Paclitaxel conjugate (Paclitaxel poliglumex, CT-2103) is already in phase II clinical trials [2] and expected enter the market in the near future [31]. Table 1 and 2 shows some polymeric prodrugs currently in the market and undergoing clinical trials respectively.

Table 1: *Examples of Polymeric Prodrugs in the Market*
(**Source:** U S Food and Drug Administration Website and Websites of Pharmaceutical Companies supplying these Drugs)

Trade Name	Technology	Company	Indication	Approval Year
Adagen®	PEGylated adenosine deaminase	Enzon	Severe combined immunodeficiency disease	1990
Oncasper®	PEGylated L-asparaginase	Enzon	Acute lymphocytic leukemia	1994
PEG-intron®	PEGylated interferon alfa-2b	Schering	Chronic hepatitis C	2001
PEG-ASYS®	PEGylated interferon alfa-2a	Roche	Chronic hepatitis B and C	2002
Neulasta®	PEGylated granulocyte colony stimulating factor analog	Amgen	Febrile neutropenia	2002
Somavert®	PEGylated recombinant	Pfizer	Acromegaly	2003

	analogue			
	of the human growth hormone			
Macugen®	PEGylated anti-VEGF aptamer	Osi-Eyetech	Age-related macular degeneration	2004
Mircera®	PEGylated erythropoetin receptor	Amgen	Anemia associated with chronic	2007
	Activators		kidney disease	
Cimzia®	PEGylated tumor necosis factor	UCB Pharma	Crohn's disease	2008
	alfa inhibitor			
Krystexxa®	PEGylated urate oxidase	Savient Pharma.	Gout	2010
Omontys®	PEGylated peginesatide	Takeda Pharma.	Anemia caused by chronic kidney	2012
			disease	
Zinostatin	Styrene Maleic Anhydride-Neocarzinostatin	Yamanouchi	Hepatocellular carcinoma	1990
Stimaler®	(SMANCS)			
XYOTAX®	Polyglutamic acid-paclitaxel	Cell Therapeutics	Non-small-cell lung carcinoma	Filed EMEA

Table 2: *Examples of Polymer Prodrugs in Clinical Development (Adapted from Reference 41 And 42)*

Trade Name	Technology	Company	Indication	Clinical Phase
CALAA-01	Polymer-cyclodextrin nanoparticle-	Calando Pharma.	Solid tumors	Phase I
	siRNA			
NKTR-105	PEG-docetaxel	Nektar	Solid tumors including hormone-	Phase I
			Refractory prostate cancer	
XMT-1001	Biodegradable polymer-camptothecin	Mersana	Solid tumors	Phase I
IT-101	Polymer-cyclodextrin nanoparticle-	Calando Pharma.	Solid tumors	Phase I / II
	Camptothecin			
NKTR-102	PEG-irinotecan	Nektar	Breast, ovarian and cervical cancers	Phase II
ProLindac®	HPMA copolymer palatinate	Access Pharma.	Ovarian cancer	Phase II
NKTR-118	PEG-naloxone	Nektar	Opoid-induced constipation	Phase II
CDP 791	PEGylated-anti VEGFR2 Fab fragments	UCB Pharma	Non-small cell lung cancer	Phase II
	as an angiogenesis inhibitor			
Paclical®	Micelles xr17	Oasmia	Ovarian cancer	Phase III
		Pharmaceutical AB		
Genexol-PM®	Micelles	Samyang	lung, ovarian, breast cancer and	Phase II
			advanced forms of Kaposi's sarcoma	

4. Targeted Drug Delivery Systems

Cancer drugs can cause enormous toxicity to normal tissues and cells [16, 24, 42, 43] and therefore the opportunity to deliver them locally creates the possibility of improving both the safety and efficacy of cancer chemotherapy [44]. The need for the development of novel cancer therapies and drug

delivery strategies that provide specific targeting of tumor cells has been continually at the forefront of medical science [45]. Drug delivery systems may either be active targeting or passive targeting [18]. Passive targeting is achieved by exploiting the “enhanced permeability and retention” (EPR) effect [2, 3, 30, 46]. Tumor microvascular endothelium exhibits elevated permeability as a result of various vascular mediators such as bradykinin and prostaglandins which induce extensive vascular permeability, coupled with lack of lymphatic drainage, macromolecules accumulate in tumor tissues for long periods compared to normal tissues [30, 46]. This is the EPR effect which is observed in almost all human cancers with the exception of hypovascular tumors [8] and depicted in Figure 2.

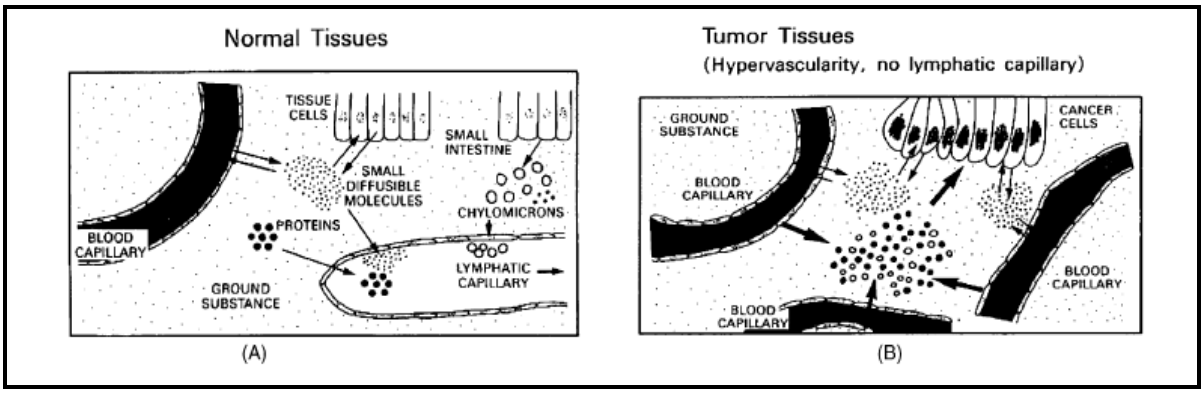

Figure 2: *The EPR Effect (Adapted from Reference 20)*

Targeted delivery is essential because of the narrow therapeutic index of most antitumor drugs and hence the urgent medical need to achieve focused drug delivery [47].

Several limitations to passive targeting such as variable vascular hyperpermeability among different tumor types and different areas of the heterogenic tumor tissue, the stagnation of the drug around the tumor tissue instead of internalization within the tumor, makes active targeting a necessary choice [2, 3]. Active targeting exploits mechanisms of receptor-ligand interactions [30] as certain proteins or receptors are over expressed on tumor cells [3]. In active targeting, a targeting moiety is used in order to direct the molecule of interest to the target in a more specific way and thus overcome the limitations of passive targeting [2, 3] by promoting uptake by cells for which it was intended for. Active targeting is independent of the EPR effect and specific interactions between the conjugates and targets can result in the active uptake of therapeutics in target tissues or cells enhancing the therapeutic effect [28].

An active targeted drug delivery offers high specificity as the ligands combine to specific receptors expressed on tumor cells and this result in a high intra-tumor accumulation and subsequent increase in intracellular concentrations of the drug. Active targeting of particulates that carry physically entrapped drugs can achieve drug delivery to target cells in vivo, thus maximizing the therapeutic efficacy of the drug and reducing its systemic side-effects [47]. To obtain a tumor-specific or a tumor cell-specific drug delivery and thus an antitumor activity of a drug, proper choice of carrier, type of linkage between the drug and carrier and proper selection of a targeting moiety and its binding procedure are very important factors [2, 48]. Extensive research has been done on targeting moieties. Practically, any molecule over expressed in the disease being targeted, either extra- or intracellularly can be exploited. Such over expressing molecules or biomarkers include the EGFR receptor, VEGF receptor, αvβ3 integrin, E-selectin, transferring receptor and folate receptor. These biomarkers are targeted by these targeting moieties such as antibodies which are directed to antigens on cell surface and peptides which selectively bind to cell surface receptors. Others include lectins and agglutinin which bind to diseased colon tissue and releases drugs at the vicinity of the diseased tissue [28].

Active targeting polymer-drug conjugates mostly undergo internalization through receptor-mediated endocytosis in which macromolecules bind to complementary receptors on the cell surface and enter the cell as a receptor-macromolecule complex in clathrin-coated vesicles, a mechanism which increases the efficiency of internalization of macromolecules more than 1000-fold compared with pinocytosis [2]. The drug is then released from the system by a specific enzymatic cleavage or by pH-controlled chemical hydrolysis. The process of an active targeted polymer-drug conjugate is depicted in Figure 3.

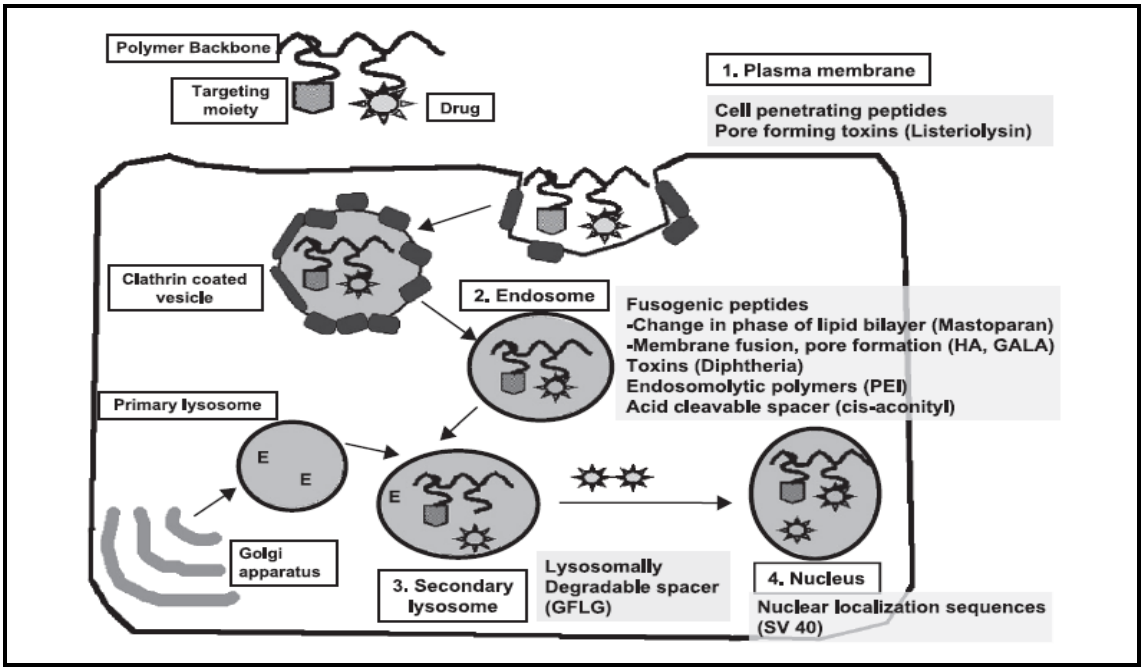

Figure 3: *Cell Internalization of Ligand Conjugated Polymeric Prodrug (Adapted from Reference 3)*

5. Antibody Conjugated Polymeric Prodrugs

Monoclonal antibodies have been used in a variety of ways in the management of cancer, including diagnosis, monitoring, and treatment of disease because of their inherent abilities to recognize and bind to tumor-associated antigens that are either exclusively expressed on tumor cells, a rarity or over-expressed as compared to normal tissue [45]. The conjugation of an antibody to a polymeric drug system confers on it selectivity in active targeting of tumor cells and improve antitumor potential of the polymer prodrug [32]. Currently, there are eleven antibodies approved for therapy listed here with the target and company in parentheses including Rituxan© (CD20; Genentech), Herceptin© (HER2; Genentech/Roche), Erbitux© (EGFR; Imclone/Lilly), Avastin© (VEGF; Genentech/Roche), Vectibix© (EGFR; Amgen), Arzerra© (CD20; Genmab), Yervoy© (CTLA-4; Bristol-Myers Squibb), Adcetris© (CD30; Seattle Genetics) [49]. Some of these antibodies targeting cancer cells such as herceptin and cetuximab have been exploited by conjugation to polymeric drugs [34, 35]. Antibody conjugation to polymers directed against tumor associated antigens opens a new area of research for scientist due to broad number of polymers which have the ability to combine to antibodies. Water-soluble polymers and their conjugates with antibodies, fragments of antibodies or oligopeptides became useful drug delivery systems suitable as carriers mainly for anticancer or anti-inflammatory agents [48]. Antibody conjugated polymeric prodrugs bind with antigen-bearing cells and the cytotoxic drug linked to the polymer is delivered directly to the tumor either externally or by absorption of the antibody conjugate rather than being absorbed systematically. The properties of the antibody will determine if the cytotoxic drug will internalize after binding with the antigens which enhances cell killing efficiency [50, 51].

In the conjugation of an antibody to a polymeric prodrug, it is important that the antibody doesn't lose its binding activity [28].

The most widely used method for the conjugation of an antibody to a polymer is the EDC Activated Conjugation Chemistry. In this method, the carboxyl group of the polymer is activated using EDC, a water-soluble zero-length cross-linker at acidic pH, forming an amine-reactive O-acylisourea intermediate which can directly react with amines [34]. Crosslinking is the process of chemically joining two or more molecules by a covalent bond. The technique, often called bioconjugation when referring to its use with proteins and other biomolecules. Crosslinking reagents (or crosslinkers) are molecules that contain two or more reactive ends capable or chemically attaching to specific functional groups (primary amines, sulfhydryls, etc.) on proteins or other molecules. Maya et al. [34] synthesized a conjugated drug polymer nanoparticle; Cetuximab conjugated paclitaxel O Carboxymethyl Chitosan using this method.

An improved method for antibody conjugation is the EDC-NHS Chemistry. *N*-hydroxysuccinimide (NHS) or its water-soluble analog (Sulfo-NHS) is often included in EDC coupling protocols. In their work, Arya et al. conjugated herceptin to a chitosan nanoparticle using the EDC-NHS Chemistry [35]. NHS and Sulfo-NHS are used to prepare amine-reactive esters of carboxylate groups for chemical labeling, cross linking and solid-phase immobilization applications. Carboxylates (-COOH) may be reacted to NHS or Sulfo-NHS in the presence of a carbodiimide such as EDC resulting in a semi-stable NHS or Sulfo-NHS ester, which may then be reacted with primary amines (-NH) to form amide crosslinks. Although NHS or Sulfo-NHS is not required for carbodiimide reactions, their use greatly enhances coupling efficiency.

In their review, Lu et al. also discussed a method for the conjugation of antibody fragments to a polymer by reacting the SH group of the antibody fragment with the maleimido group containing monomers of various spacers between the double bond and the maleimido bond [28].

Antibody conjugated polymeric prodrugs have some functional similarities to antibody drug conjugates. Its greater benefit is that the encapsulated drug is protected from immune degradation as well as it enables a fairly slow release of the drug systematically which offers a high drug efficiency as well as an extremely low toxicity.

A typical example of an antibody conjugated polymeric prodrug is Certolizumam pegol, marketed as Cimzia® by UCB. It is a novel PEGylated anti-TNFα monoclonal antibody for the treatment of rheumatoid arthritis [2].

6. Challenges of Antibody Conjugated Polymeric Drugs

There have been a number of questions raised about the targeting ability of antibody conjugated delivery systems. The main aim of a drug delivery system is to reach only one target organ while sparing all other organs. In his literature, Al-jamal [52] emphasized that this is rarely the case as most delivery systems reach other organs often at even higher concentrations than the tissue/organ of interest thus undermining the aim of drug targeting. He went on further to explain that most receptors are present not only in tumor cells but also healthy cells and thus active targeting drug delivery system obviously has shortcomings which needs to be addressed. In their review, Kwon et al. believed that targeted delivery is a challenge that may not be achieved in the near future [53]. They emphasized that over expression of receptors has little to do with targeted delivery because over expression on cancer cells mean that normal cells also express the receptors and since normal cells is much larger than the number of cancer cells, it is reasonable to consider that most of the ligand is captured by normal cells. They further went on to explain that drug release can occur before reaching the target site and that reaching the tumor site is not the same as improved delivery as this is not the

endpoint of the targeted drug delivery. Zhang et al. in their review, Advanced materials and processing for drug delivery: the past and the future also raised concerns about unexpected drug release associated with polymer-drug conjugates [29]. The large size of antibodies which impedes its movement as well as their immunogenicity also limits their use in targeted delivery systems [3].

The use of in vitro screening anticancer studies instead of in vivo studies and the relatively complex nature of characterizing polymer-drug conjugates posses some challenges in the study of targeted polymeric prodrug delivery systems. In their review, Francesca Greco and Maria Vincent [31] wrote that the usefulness of in vitro studies of anticancer activity polymer-drug conjugates is debatable as they rely on the accumulation in the tumor via the EPR effect, which can be observed only in vivo models. They also emphasized that that the conjugation of a drug to a carrier is often a random process and although optimization of reaction conditions ensures a good degree of batch-to-batch reproducibility, the point of attachment of the drug within the chain remains in most cases not controllable and not directable.

7. The Future of Cancer Therapy with Antibody Conjugated Polymeric Prodrugs

Although quite a number of works have been done in the development of antibody conjugated polymeric drugs, most have not been accepted for clinical use. The concept of targeted delivery using this system still needs to demonstrate the clinical successes that are theoretically anticipated. Most in vitro studies have shown great results but certain in vivo challenges such as non specific uptake by the liver, kidney and spleen, shared antigen cross reactivity, a significant loss in the biological activity of the antibody and poor penetration into solid tumors needs to be addressed.

Scientists should put in more effort in the nanosizing of these antibody conjugated polymeric drugs [54] as this will offer a high drug loading efficiency, constant drug delivery as well as a high drug bioavailability. Nanoparticles are one of the most promising anticancer drug vectors because they can be delivered to specific sites by passive targeting or active targeting [55] and they may also protect a drug from degradation, enhance drug absorption by facilitating diffusion through the epithelium, modify pharmacokinetic and drug tissue distribution profile and/or improve intercellular penetration and distribution [56]. Nano-sized particles accumulate in solid tumors at much higher concentrations than in normal tissues or organs due to the EPR effect [18].

Challenges such as premature drug release can be alleviated if these nanoparticles are properly modified as shown in recent studies where polymer micelles have shown that drug release can be delayed until they are endocytosed by cancer cells, if either the shell or core of polymer micelles is crosslinked [53]. The construction of nano metal oxide frameworks illustrates original concepts able to improve drug loading and/or to reduce the burst release [57].

In addition, antibody-directed enzyme prodrug therapy (ADEPT) can be exploited [30]. In this therapy, an enzyme capable of converting a non-toxic prodrug into a potent cytotoxic drug is covalently attached to a tumor selective monoclonal antibody where the potent cytotoxic drug is only released at the tumor site. This minimizes premature drug release and its associated toxicities.

The challenges associated with the large size of antibodies can be addressed using antibody fragments such as the HPMA copolymers synthesized from polymerizable Fab' antibody fragments based on the OV-TL16 antibody which demonstrated a superior tumor directed toxicity as compared to non-targeted conjugates [28].

More studies needs to be done on polyclonal antibodies which has least been exploited in antibody conjugated polymeric prodrug systems. Not only are they cheap to produce as compared to

monoclonal antibodies, polyclonal antibodies they are capable of recognizing multiple epitopes on any one antigen hence can be used for tumor cells expressing more than one antigen.

8. Conclusion

Current measures used in the treatment of cancers although effective come with its related toxicities and drug safety concerns. A targeted delivery using antibody polymeric prodrugs is one of the approaches which can be used in addressing these concerns due to its selectiveness and hence preventing the undesirable side effects of cytotoxic drugs as well as reducing the incidence of drug resistance associated with current therapy. A polymerized drug is protected from enzymatic degradation and prevents immune responses and hence a better therapeutic outcome. Challenges affecting the use of antibody conjugated polymeric drugs such as the specificity of this delivery system can be addressed by measures such as the nanosizing and modifications of these systems which offer a better drug loading, better targeting, internalization and enhanced drug properties.

A better understanding of the human physiology and tumor biology such as identification of signaling pathways specific for macromolecular therapeutics and manipulation of tumor microenvironment are the essential tools to indeed develop a very potent targeted delivery system. With current progress in research and clinical trials, it is my believe that the future of cancer therapy will be these forms of targeted delivery such as antibody conjugated polymeric prodrugs.

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