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Targeted Drug Delivery: Overcoming Barriers through the Design of Novel Delivery Vehicles

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ABSTRACT

In this overview, recent developments on stimuli sensitive systems for targeted drug delivery and various delivery routes have been described. Novel technologies used to address challenges associated with the delivery of large drug molecules along with clinical applications have been presented.

Keywords: Drug delivery systems (DDSs); Controlled release; Targeted delivery; Responsive systems

INTRODUCTION

For centuries, chemically synthesized drugs and antibiotics have been discovered and developed continuously. Especially in recent years with the discovery of powerful drugs, promising treatments have been developed for various cancer types. However, it was realized later that these drugs have side effects due to relatively nonspecific nature of the mechanism of chemotherapeutics. Targeted drug delivery has become an attractive area of research during the last decade, as can be observed from increased number of publications related to targeted delivery over years (Figure 1). Most drugs do not have special affinity towards disease sites in the body, hence they compromise healthy cell function nonspecifically, and ultimately cause toxic side effects [1]. Toxicity problem arises from considerably high amount of drug concentration administered to reach its therapeutic efficacy. Selective targeting can address toxicity issues, and decrease side effects originating from the normal cell-drug nonspecific interactions [2].

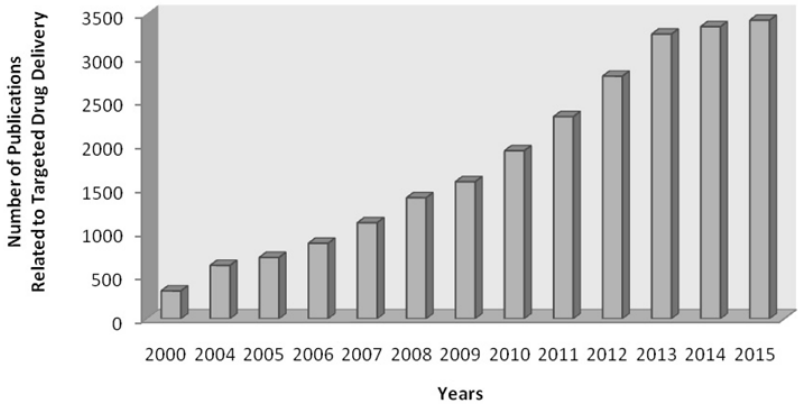

Figure 1: Distribution of publications by years. The number of targeted drug delivery related articles has increased in years according to search results taken from Web of ScienceTM database (January 15, 2015).

One of the challenges related to targeted drug delivery is the lack of feasible and practical administration strategies of drugs [2]. Other challenges are associated with the variety of barriers in the body from organ-to-subcellular level. These barriers can be overcome through efficient therapeutics transport strategies, proper sub-molecular target epitopes and specific target choices [3]. Physiological variables, such as blood flow for intravenous administration of drugs and tissue architecture, along with physicochemical parameters including carrier geometry, avidity, composition and functionalization should be controlled for effective targeting of desired cells or tissues [3]. Human genome project contributed to understanding diseases at molecular level [4]. This progress will allow further developments in the selection of the specific targets related to various diseases.

Various bioactive molecules from pharmaceuticals to nucleic acids, and various delivery vehicles, such as liposomes, micelles or stimuli-responsive hydrogel systems have been considered as illustrated in Figure 2. External stimuli such as ultrasound, magnetic field, environmental pH, and temperature changes have been chosen depending on the type of the delivery vehicle used. With the use of an appropriate drug administration, defined by the targeting site and the nature of the delivery vehicle, drugs may reach to specific tissues, cells of interest, or cellular compartments including nucleus and organelles.

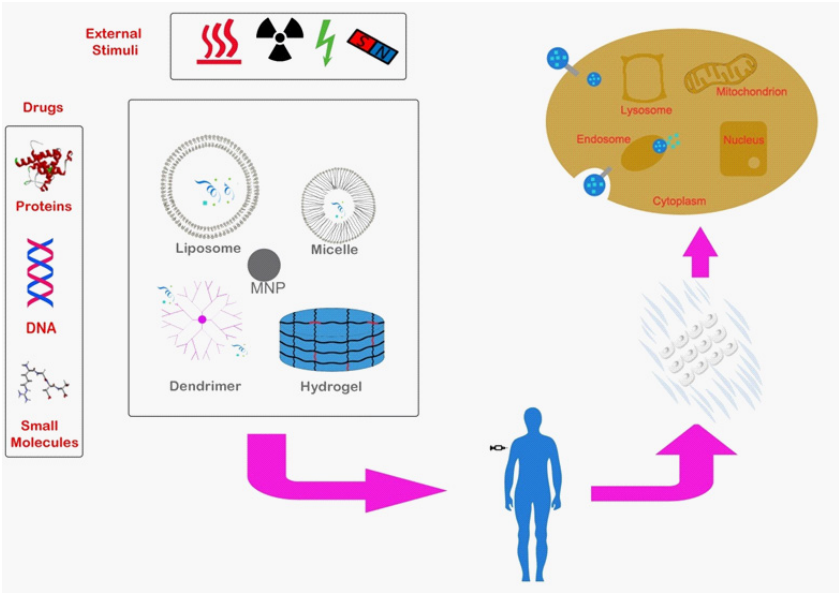

Figure 2: Schematic representation of responsive drug delivery systems. Drugs including bioactive molecules such as small drug molecules, peptides, therapeutic proteins and DNA, may be carried by delivery vehicles and release can be induced by external stimuli such as irradiation, heat or pH. These intelligent delivery systems target various regions in cells and tissues as cell surface receptors, nucleus or organelles.

DRUG DELIVERY VEHICLES

Conventional Mechanisms: Diffusion-controlled and Solvent-activated Systems

Bioactive molecules incorporated with delivery agents have been used more than 50 years to treat various diseases, and those systems contributed to the development of promising targeted therapeutics with controlled release properties [5]. The aim was to deliver the drug at a specific rate for a certain period of time [6]. These studies enabled us to control the temporal and spatial release of the drug, to prevent physiological elimination of the drug, to reduce immune response of the host, and to maintain normal pharmacokinetic properties of the drug [7,8]. Identifying and understanding the mechanism of the release process is important in controlled release systems [7]. Conventional drug delivery mechanisms, in which diffusion is the rate-limiting step, are basically classified as diffusion-controlled (monolithic devices) and solvent-activated (swelling- or osmotically controlled) mechanisms [5].

Diffusion-controlled devices are categorized as matrix- and reservoir-type systems [9]. In matrix-type systems, drug is dispersed or dissolved in a nonswellable or swollen polymer material, which is not degradable, and the release occurs by diffusion from the preloaded matrix [5,7,9]. Drug is separated from a polymer membrane which serves as a diffusional wall to yield

drug flux in a reservoir-type system. An osmotic pump device is a subgroup of reservoir-type systems; a small hole is inserted in these water permeable membrane devices, where the drug displaces through the slit as it dissolves in water [7,9].

Solvent-activated mechanisms consist of three steps: solvation of the molecules at the solid-liquid interface, severance from the solid surface, and diffusion through the bulk solution. Embedding solid drug particles into matrix systems are complicated, and makes the design step crucial [5,9].

Novel Formulations for Drug Delivery

For effective formulation and delivery of drugs, it is essential to design an appropriate system that considers proper delivery pathway [8]. Numerous DDSs have been investigated up until now. These contain nanocarriers such as lipid-based liposomes and micelles, implantable delivery systems, polymeric microspheres, and biologically adhesive delivery systems [8,10]. Nanocarriers can be defined as drug reservoirs at nanoscale circulating within the blood, where plasma clearance rate is less than that of the free drug. Liposomes, dendrimers, micelles, solid lipid and polymer nanoparticles, and polymer-drug conjugate with drugs have been widely used for controlled drug release studies [7]. Lipid-based systems were first described in 1965 as swollen phospholipid membrane model. Enclosed structures of bilayered phospholipids were obtained, and named as liposomes [11]. Next, liposomes have been found suitable for the delivery of both water-soluble and water-insoluble drugs due to their inner aqueous part and hydrophobic domain of the phospholipid bilayer [10,12]. Liposomes have been known as potent drug carriers for more than twenty years for being totally biocompatible [10]. However, classical liposomal carriers can be rapidly removed from the blood by mononuclear phagocyte system or reticuloendothelial system. To prevent the elimination of liposomes and the entrapped drug molecules, pre-dose of empty liposomes were administered as a strategy to block phagocytic cells [11]. Drug dependency of detainment in liposomes and fast leakage of water soluble drugs are some of the limitations of liposomal delivery systems [11,13]. Micelles, including polymeric micelles, are colloidal dispersions which are mostly smaller delivery vehicles in proportion to liposomes (50-1000 nm), with a size distribution of 5-100 nm range [10]. Micelles can also carry hydrophobic drugs due to their amphiphilic nature [13,14]. Micelles and liposomes can extravasate from circulation through the neoplasms which facilitates the delivery of drug specifically to tumors [14].

Implantable vehicles have also been extensively considered for *in vitro* and *in vivo* applications. They are advantageous for providing prolonged drug release rate, ease of construction, and local delivery. The risk of systemic side effects can be reduced by using local delivery approach; however this approach requires injection or surgical implantation of the vehicle. Gels and microspheres are commonly used as injectable implants, whereas sheets/films, foams and scaffolds have been preferred for surgical implants. Especially, nondegradable polymers have been frequently considered as implantable materials. Generally, miniscule devices like infusion

pumps and catheters that deliver drugs into targeted sites are used as surgical implants. For example, infusion pumps for insulin delivery used in the treatment of diabetes are commercially available for clinical use [6].

A significant amount of DDSs have been synthesized in the form of microparticles. These effective carrier particles are prepared from various synthetic and natural monomers such as poly-ethylene glycol (PEG), acrylamide(AAm), chitosan, hyaluronan, dextran, cellulose, pullulan, and alginate [15]. Emulsification technique can be applied to generate microparticles, wherein water-in-oil or oil-in-water system is employed to produce microparticles as reservoirs for the therapeutic molecule. Water-soluble polymer droplets can be suspended in oil phase such as cyclohexane, hexane, and mineral oil to generate microdroplets, which are then retrieved from oil phase [15,16]. The organic solvent in oil phase interferes with the protein stability in biological systems, thus aqueous emulsion method, based on polymer–polymer immiscibility arising with many combinations of water-soluble polymers (e.g. combinations of PEG-dextran), or water-in-water emulsion, has been employed to produce biocompatible microspheres [17,18].

Responsive and Targeted Systems for Drug Delivery

Release of a molecule in response to a specific stimulus such as pH, temperature, or protease provides opportunities to tailor site specific therapies [19]. Responsive materials would allow for the control on the dose, timing, and period of the drug release [20]. Hydrogels have been used widely for "intelligent" delivery of therapeutic substances [21]. Among these systems, administration of pH-sensitive hydrogels is particularly advantageous since different regions of the body have variable pH conditions such as gastrointestinal tract, vagina and blood vessels [22,23]. For example, a visible-light-induced pH-sensitive hybrid hydrogel system was designed as a drug carrier for controlled release of the anticonvulsant drug pregabalin (PGB), recently. In this study, poly(methacrylic acid-g-ethylene glycol) P(MAA-g-EG) was selected as a hydrophilic and pH-responsive constituent, and crosslinked styrene–butadiene–styrene (SBS) was included into the network due to its hydrophobic properties. *In vivo* implantation of the visible-light-cured pH-responsive hydrogels into rabbits resulted in no significant levels of erythema and edema (Figure 3a,3b). Furthermore, these hydrogels conserved their integrity during swelling and PGB release from the network (Figure 3c) [22]. pH-responsive biomaterials have also been used to target tumor site [22,24]. The vascular network of tumors is often inadequate to provide sufficient oxygen and nutrition for the growing mass of tumor cells. These cells produce lactic acid under hypoxic conditions, and hydrolyze ATP that forms acidic side-products. Therefore majority of solid tumors have lower extracelluar pH (pH <7.2) than the healthy tissues and blood (pH 7.5). In a previous study, hydrogel nanoparticles self-assembled from conjugates of pullulan derivative and sulfadimethoxine (PA/SDM) were used to release Adriamycin at lower pHs [24]. Thermoresponsive carriers have also been considered as a site-specific targeting approach. Many polymers reveal a temperature-responsive phase-transition due to the presence of hydrophobic groups, such as methyl, ethyl and propyl groups. Poly(N-iso- propylacrylamide) (PNIPAAm)

and poly(N-isopropylacrylamide) (PIPAAm) are well-known examples of these polymers [25]. pH and temperature alterations are usually employed to induce behavioral changes. In addition, functional materials that are stimuli-responsive such as ultrasound, electromagnetic radiation, near-infrared light (NIR), ionic strength, redox potential have been explored in recent studies, and have been considered as drug carriers [5,20]. These systems can be utilized for imaging of drug loaded carriers [20].

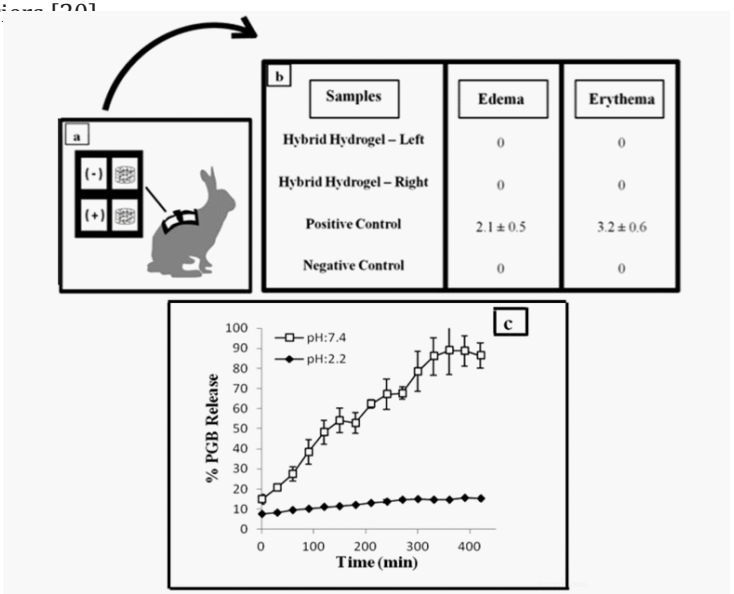

Figure 3: PGB release results from visible-light induced pH-responsive P(MAA-g-EG) hydrogels and *in vivo* implantation results for skin irritation. (a) Schematic representation of intracutaneous reactivity assay sites, (b) results of intracutaneous reaction and (c) Release behavior of PGB from a visible-light cured pH-responsive P(MAA-g-EG) hydrogel was examined at pH 2.2 and pH 7.4, indicating the increment in PGB release in higher pHs [22].

Inorganic materials such as metal, metal oxide, and semiconductor, as well as minerals and silica comprise an important type of the nanoparticles. Nanoparticles synthesized with these materials frequently hold unique electromagnetic and optical features. For instance, gold nanoparticles have specific electromagnetic and optical features, and hence they have been used as contrast agents for optical imaging. Gold nanoparticles display remarkable near-infrared light absorption in consequence of coupled plasmon resonance. Magnetic nanoparticles are another type of inorganic nanoparticles that usually consist of iron oxides (magnetite and maghemite). In the presence of magnetic fields, the magnetic moments of these superparamagnetic nanoparticles align and reach saturation. This property makes them detectable by magnetic resonance imaging (MRI), and hence they have potential to be used as vehicles for diagnosis and treatment of many diseases including cancer [26].

Organic nanoparticles are another type of nanostructures made of numerous biodegradable polymers, materials with high protein content, or lipids. Small molecules, peptides, proteins, and nucleic acids can be encapsulated in these nanoparticles to ensure controlled release and targeted delivery. Correspondingly, magnetic nanoparticles encapsulated in polymer nanoparticles to enhance circulation and targeting, can be used as clinical imaging contrast agents, as well as targeted delivery agents [19]. The system may be designed to respond more than one local stimulus when it is desired [21]. In a recent study by Nazli et al., magnetic iron oxide nanoparticles (MIONPs) were coated to create matrix-metalloproteinase (MMP)-sensitive and targeted nanocarriers for intracellular delivery of doxorubicin into cancer cells (Figure 4). Integrin-targeted and MMP-sensitive PEG hydrogel scaffolds were used to coat magnetic iron oxide nanoparticles. This functional coating promoted intracellular targeting of the drug, as integrins and MMPs are overexpressed in tumor cell surfaces and microenvironment, respectively. Cancer cells treated with doxorubicin loaded-targeted nanoparticles exhibited significantly lower survival than controls exposed to doxorubicin loaded-nontargeted nanoparticles [19].

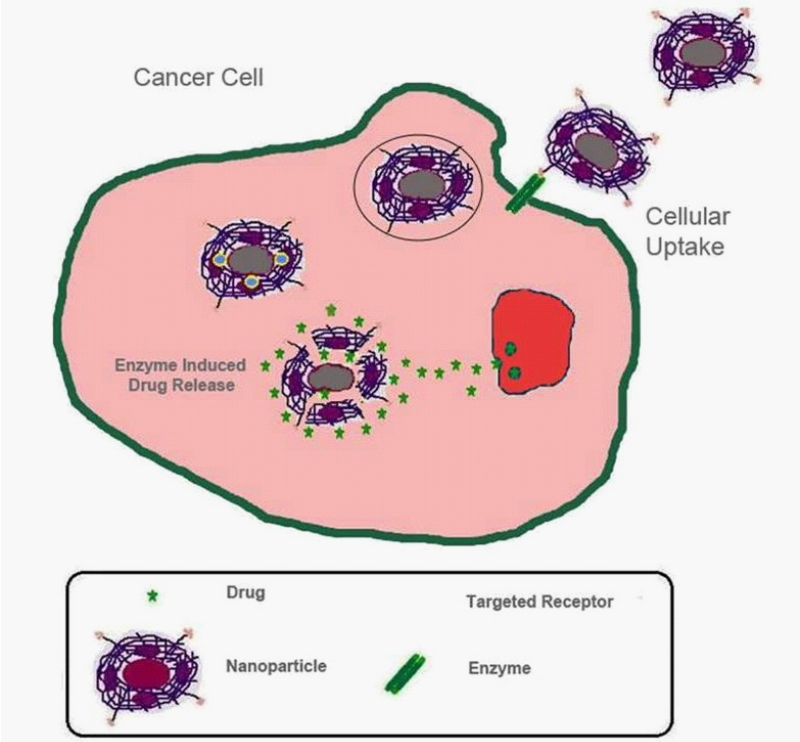

Figure 4: Schematic representation of the intra-cellular delivery of anticancer drug doxorubicin into tumor cells by targeted nanocarriers. Via receptor-mediated endocytosis, arginine-glycine-aspartic acid-serine (RGDS) ligand of the targeted nanocarriers binds to αvβ3 integrin, and internalization of nanocarriers can be achieved into tumor cells. Following the degradation of MMP-sensitive domains in PEG hydrogel coating of targeted nanocarriers, loaded doxorubicin is released into the cytoplasm of cancer cells [19].

Majority of conventional drug delivery systems can be modified to achieve targeted delivery. Dextrans, PEG, polyacrylamides, and albumin are usually considered for conjugation of drugs [6]. For example, PEGylation strategy was used to increase stability and bioactivity of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). This protein-based therapeutics was loaded into biodegradable poly(lactic-co-glycolic acid) (PLGA) microspheres, and sustained release was achieved from the network [27]. Drugs can be also conjugated with polymeric dendrimers which are hyper-branched tree like structures with drug entrapment features. PAMAM-G4 dendrimer conjugation enhanced the efficiency of paclitaxel 10 fold in human ovarian cancer cells (A2780) compared to its free form [5,28].

Recently, fibrin-binding peptide modified systems attracted attention for the targeting of thrombi. Cysteine–arginine–glutamic acid–lysine–alanine (CREKA) peptide was conjugated to nanoparticles through a maleimide (Mal)–thiol reaction which resulted in better targeting of glioblastoma multiform (GBM) cells compared to commercial PTX (Taxol®) or conventional PTX nanoparticles [29,30].

TARGETING STRATEGIES

Intracellular Delivery

Intracellular delivery of pharmaceutical agents like proteins, antibodies and drug loaded nanocarriers are used to ensure that the therapeutic action is specifically introduced to nucleus or specific organelles. Cell nuclei targeting for gene therapy, lysosomal compartment targeting for lysosomal enzymes and mitochondria targeting for proapoptotic drugs are among the intracellular targeting routes (Figure 5) [10]. One of the challenges associated with the intracellular transport of active molecule is the presence of hydrophobic cell membrane which restricts the entrance of big molecules, such as DNA and proteins. The receptor-mediated endocytosis is utilized for intracellular transport; however, particles taken into the cell via endocytosis can be entrapped in endosome, and then lysosome for subsequent enzymatic degradation. Hence, only a small amount of unaffected substance is found in the cytoplasm, validating the problems in the transformation of *in vitro* studies into *in vivo* about limited bioavailability of the drug. Invasive and noninvasive methods have been used to bypass endocytic pathway, deliver membrane-impermeable molecules, and enhance drug efficacy. Electroporation and microinjection are among the invasive methods that could damage cellular membrane. Alternatively, drug can be delivered through a noninvasive method with pH-sensitive liposome, where the acidic environment in endosome leads to destabilization of endosomal membrane and release of loaded drug [31,32]. Liposomes and micelles can be modified with pH-sensitive domains to deliver the drug loaded carriers into individual organelles due to the alterations of pHs from 4.5 to 8 in different organelles [33]. pH-sensitivity of the liposome can be achieved by the presence of fusogenic lipids, including unsaturated 1,2-dioleoyl-phosphatidylethanolamine (DOPE) or oleyl alcohol [34]. Antibody-modified pH-sensitive liposomes and polymeric micelles demonstrated successful delivery of

antitumor drugs, with improved targeting capacity and intracellular availability due to facilitated uptake [35]. For instance, PEG-poly(aspartate hydrazine adriamycin) micelles were developed to release loaded drug at acidic pH typical for endosomes, and have potential to be used for cancer cells [36]. In a recent study by Torchilin et al., PTX-containing micelles were synthesized with PEG-PE and Lipofectin®, a membrane-destabilizing agent, where increased intracellular delivery of PTX, and improved anticancer activity of the drug was demonstrated [37].

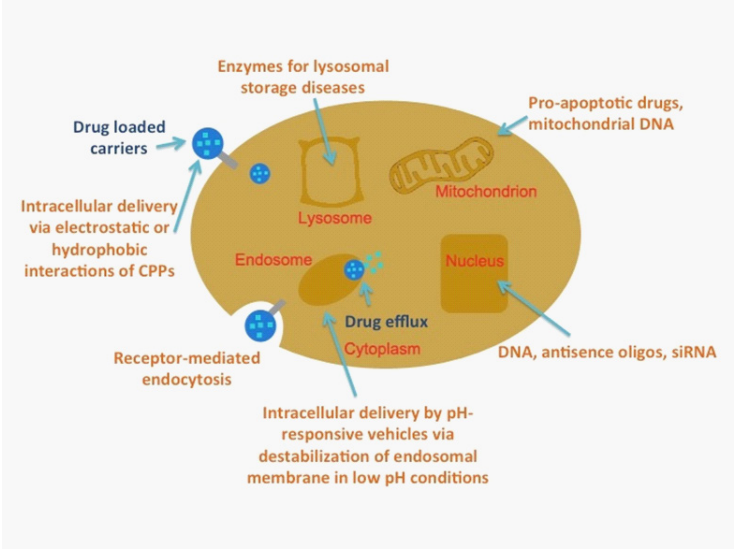

Figure 5: Schematic representation of intracellular targets and possible intracellular drug and DNA delivery routes/ methods. Intracellular targets can be named as the nucleus, mitochondria or lysosome. According to the specific organelles od interests, various targeting strategies can be utilized, such as direct, receptor-mediated and CPP-mediated intracellular delivery, as well as the usage of pH-sensitive carriers for internalization of drugs [10].

Another novel intracellular delivery material developed involves cell-penetrating proteins or peptides (CPPs). Several proteins and peptides are known to traverse through cellular membrane, and deliver their cargo molecules into the cytoplasm or nucleus. Cellular delivery with CPPs, using short sequences of proteins with less than 20 amino acids, is an effective method for various cell types [38]. Lysine (Lys) bearing amphipathic helical peptides [39], the human immunodeficiency virus type1 (HIV-1) transcriptional activator TAT proteins [40], and arginine (Arg)-rich peptides have been widely studied types of CPPs [41]. Intracellular delivery of large molecules can be overcome via energy-dependent macropinocytosis [42], where CPPs or CPP-conjugated small molecules may penetrate into the cells via electrostatic interactions and hydrogen bonding [43]. Although CPPs are capable of delivering a variety of biological molecules, like proteins, peptides, DNAs and nanoparticles into different cell types, the use of CPP is limited by their nonselective internalization into cells. In a recent study, novel cancer-homing CPPs were identified to target GBM as selective transporters. Higa et al., screened random peptides to find CPPs bearing affinity

for human U87MG GBM cells. The authors demonstrated that the GBM-selective CPPs induced apoptosis, suggesting successful delivery of therapeutic molecules into human GBM cell line via CPPs [44].

Targeting Mitochondrion

Mitochondrion is an important organelle for targeted intracellular delivery of pharmaceuticals. Mitochondria are involved in the oxidation of fatty acids, the synthesis of steroid hormones, citrate cycle, and ATP production. Various disorders, such as cancer, diabetes and neurodegenerative diseases have been linked to mitochondrion dysfunction [10]. To date, PTX and many other drugs have been known to induce mitochondrion triggered apoptosis [45], and these have been used for cancer treatment. Mitochondrion targeted drug/gene delivery is promising, and mitochondrion targeting has been an attractive strategy for several cancer therapies to increase the efficacy of drugs [46-49]. For example, in a previous study, tumor cell proliferation was suppressed via mitochondrion targeting of a drug that acted on calcium channels [50]. In another study, mitochondrial phenotype of three different oral squamous cell carcinomas (OSCC)-derived cell lines were characterized and the cytotoxic effect of the metabolic drug dichloroacetate (DCA) in carcinoma cells was demonstrated for the treatment of the most frequent oral cavity carcinoma [51,52].

Targeting Lysosome

Several diseases, known as lysosomal storage diseases resulting from the insufficient amount of various lysosomal enzymes, have been treated by administration of exogenous enzymes, including glucocerebrosidase, glucosidases and phenylalanine ammonia lyase [53]. This therapy is limited with the short life time of the enzymes in circulation, and considerably low transport into lysosomes [54]. pH-sensitive liposomes are promising for intracellular transport of exogenous enzymes. After endocytosis and endovacuolar membrane fusion of liposome-encapsulated enzymes, the cargo can be released intracellularly with the effect of acidic endosome pH [10]. Direct infusion of purified enzymes, such as hexoseaminidase [55], and ceramidetrihexosidase [56], to treat Tay-Sachs or Lesh-Nyhan syndromes were unsuccessful, whereas the use of liposome-immobilized enzymes demonstrated better results [57]. In a recent study, weakly basic substances were found to be efficient molecules for extensive sequestration in acidic lysosomes [58,59]. For example, in the neutral cytosol environment with appropriate pKa values, basic molecules known as lysosomotropic compounds, exist in un-ionized, membrane-permeable form, whereas after permeation through organelle membranes and encountering acidic luminal pH, the compound remains at its ionized, membrane-impermeable form. It was shown that several cancer cell lines have faulty acidification of lysosomes [60], and this phenomenon resulted in altered drug concentrations in the cytosol with reductions in lysosome-to-cytosol pH-gradients. For these cells, reduced capacity of lysosomal sequestration was observed, followed by higher cytosolic concentration, elevated drug interaction with cytosolic or nuclear targets, and increased

toxic effects. This difference in drug distribution between normal and cancer cells (Figure 6), resulted in improved toxic effects towards cancer cells, and formed the basis for intracellular distribution-based (IDB) drug targeting [58]. Due to the lysosomotropic potential of the large number of anticancer drugs, IDB drug targeting is promising, provided that lysosomotropic properties of the anticancer agents are optimized [58].

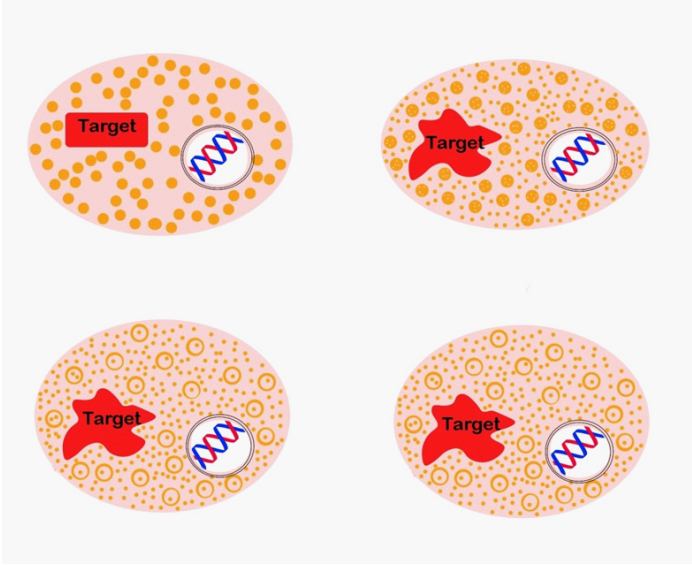

Figure 6: Schematic overview of the intracellular distribution-based (IDB) drug targeting platform. IDB drug targeting benefits from the differences in intracellular distribution behavior that exist for lysosomotropic drugs in cells with low (normal) and increased lysosomal pH (cancer). Drugs (represented as red dots) with lysosomotropic properties will be extensively separated in lysosomes of normal cells and will have less interaction with cytosolic targets (top left cell). The same lysosomotropic drug will localize differently in cancer cells due to increased lysosomal pH (top right cell). Specifically in cancer cells, the concentration of the lysomotropic drug in the cytosol will concomitantly increase and the concentration in the lysosomes of cancer cells will be reduced. The elevated cytosolic drug levels allows for greater interaction with targets and an increased therapeutic response. Anticancer drugs without lysosomotropic properties will not differentially localize in normal and cancer cells regardless of lysosomal pH status (lower cells) and there will be no effects on drug-to-drug target interactions [58].

Other Targeting Strategies

Besides endocytic pathways, such as phagocytosis and pinocytosis, the direct cytosolic delivery can be mediated by nonendocytic penetration involving the diffusion of nanocarriers through cell membrane [61,62]. This transport of nanocarriers would prevent lysosomal degradation of active drugs, and would also contribute to the rapid and high amount of drug deposition into the cytosol [63,64]. To date, intracellular delivery of various nanocarriers have been achieved, including carbon nanotubes [62] and lipid-coated liquid perfluorocarbon nanoparticles [64]. In a recent study

novel shell-crosslinked hybrid nanocapsules (HNs) based on nanoemulsion-templates, capable of penetrating across the cell membrane into the cytosol, were stabilized by both biocompatible beta-lactoglobulin (b-LG) and egg phospholipid (EPC) [65]. Antitumor activity of PTX drug was improved both *in vitro* and *in vivo* when these nanocapsules were used as a delivery vehicle. This finding demonstrated that *in vitro* antitumor activity of PTX was increased due to both high PTX-loading, and enhanced internalization into cytosol (Figure 7). Another cytoplasmic drug/gene delivery pathways that bypass the lysosomes is the caveolae-mediated uptake which is both valid for caveolin I protein expressing cells and other cell types possessing similar properties such as the formation of lipid rafts [66-68]. Photochemical internalization is the other novel approach that uses light activation of a photosensitizer drug incorporated into the endosomal membrane. This approach allows for the cytoplasmic escape of the endocytotically captured drug, followed by the induction of the endosomal membrane degradation. With this approach, more than 100-fold increase was observed in the biological activity of the drug [69].These findings are promising and show that with further understanding of diseases at a molecular level, more efficient therapeutic strategies will be developed through the targeting of other specific organelles.

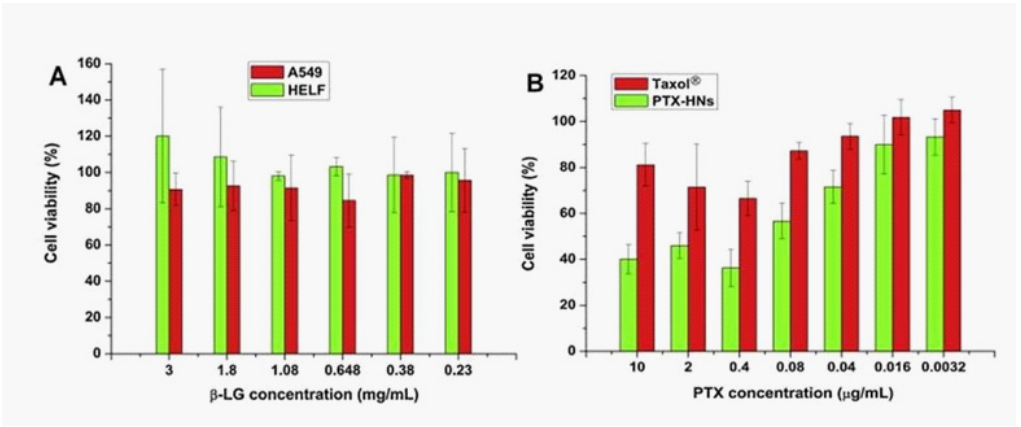

Figure 7: In vitro cytotoxicity results for HN internalization by direct cytosolic delivery. Cell (A549 and HeLa cells) viability results after incubation with (A) blank HNs and (B) Taxol® (free-PTX)and PTX-HNs for 24 h at 37°C [65].

Receptor-Mediated Transport

To treat neurological disorders such as multiple sclerosis or Alzheimer's disease, different biologics including recombinant enzymes and monoclonal antibodies (mAbs) have been developed. However, due to the limitation related to blood-brain-barrier (BBB), there is a limit both on the amount of drug uptake by brain, and on the activity of the biologics [70]. Endogenous receptor-mediated transport (RMT) systems are promising to overcome BBB. These systems benefit from the vesicular trafficking to deliver different biologics across BBB endothelium. Among BBB shuttle routes, RMT is responsible for the delivery of transferrin, insulin, leptin and other types of proteins, using the vesicular trafficking machinery of brain endothelial cells [71,72]. RMT is the ideal BBB

delivery route for various biologics, as monoclonal antibodies, recombinant proteins, RNA, DNA and nanomedicines [71,73]. Improved transport of biologics into the brain through RMT could be achieved by modification of pharmaceuticals with appropriate targeting ligands. Among the widely studied and well-established BBB RMT targets are: transferrin receptor, insulin receptor, and low density lipoprotein receptor [74]. Recently, RMT-targeting vectors have been engineered, and mechanistic details of RMT binding and intracellular trafficking have been investigated for improved brain penetration [74]. The fragment crystallizable (FC) region-5 antibody and viral coat peptide consists of 29 amino acids acquired from the rabies virus glycoprotein (RVG29) have been considered as novel alternative RMT-targeting vectors, that have the capability to pass the BBB *in vivo* [74]. The impressive therapeutic outcomes in animal studies with the use of RMT targeting strategies suggest that receptor-binding antibodies or ligand mimics are promising and could be considered as RMT-targeting vectors. Cinical research is underway and has been conducted by several companies, such as ArmaGen Technologies and Angiochem [74]. According to recent results about the transport of anti-transferrin receptor monoclonal antibodies (anti-TfR mAbs) into the BBB, limited transcytosis into the brain parenchyma was observed despite substantial binding and endocytosis into BBB endothelial cells [75-78]. Predominant localization of radiolabeled anti-TfR antibodies on brain capillaries, after both in situ brain perfusion and iv injection was observed in both rats and mice [75-78]. These observations suggested that monoclonal antibodies were trapped in the brain endothelial cells upon endocytosis.

ROUTES OF DELIVERY

Therapeutic dose, length of treatment, processing conditions on the bioactivity of drug and feasibility will contribute to the decision about the administration route of drugs [8]. The most common pharmaceutical delivery routes are oral [5,20], transdermal [12], parenteral, nasal and ocular routes [8]. Oral delivery is the most common drug delivery route due to the ease of application and low cost. As drugs follow the same route with nutrients in oral delivery, they encounter many boundaries that decrease pharmaceutical bioavailability and stability until their absorption [5,8]. Parenteral delivery of drugs is commonly managed through intravenous, subcutaneous, and intramuscular injections [8]. Compared to oral delivery, parenteral route evades the gastrointestinal tract by direct injection, intravenously or interstitially, and is faster. This route is also advantageous for hydrophobic substances [5]. For instance, Zoladex® and Lupron® depots (leuprorelin acetate), are commercially available, FDA approved, injectable parenteral delivery machines for prostate cancer therapy [8,14]. Lupron® formulation consists of PLGA microspheres. This drug reservoir releases drug for a month and degradation of whole polymer occurs within six weeks after injection [8]. For the case of transdermal administration, various techniques including chemical alteration, ultrasound, iontophoresis and electroporation have been studied to overcome nonviable epidermis barrier; however, limited success was achieved. Microneedles have also been developed to overcome epidermis barrier for improved drug delivery as a promising and minimally invasive method [12]. For the fabrication of

microneedles, silicon wafer, stainless steel and biodegradable microneedles were used. The drug was loaded into a polymer solution such as PLGA, dextrin or chondroitin sulfate to synthesize self-degrading microneedles. These microneedles have also been considered to transport vaccines for melanoma cancer [79], where immunogenicity of the vaccine was improved by encapsulating the antigen into an albumin matrix.

CONCLUSION

Design of stimuli sensitive delivery systems has become more important, and the research in this field has accelerated significantly. These delivery systems have demonstrated promising results, and have potential to reduce the side effects of drugs. In the future, further developments in biomaterials/regenerative medicine will address challenges associated with nonspecific drug-cell interactions and toxicity issues through novel targeting strategies towards specific tissues and organelles.

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