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An Overview: Engineering pH-Sensitive Polymeric Nanoparticles for Enhanced Intracellular Delivery of Anticancer Drugs in Hypoxic Tumors

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ABSTRACT

Hypoxic tumor microenvironments pose significant barriers to effective chemotherapy. We developed pH-sensitive polymeric nanoparticles capable of releasing doxorubicin selectively in acidic conditions typical of hypoxic tumors. Nanoparticle characterization confirmed size uniformity, pH-responsiveness, and stability. In vitro and in vivo studies demonstrated enhanced cellular uptake, drug release under acidic conditions, and superior tumor growth inhibition compared to free drug formulations. Our platform offers a promising strategy for overcoming hypoxia-associated chemoresistance.

Keywords: Cancer Therapy, Drug Delivery, Hypoxic Tumors, Doxorubicin, Ph-Sensitive Nanoparticles, Tumor Microenvironment

1 Introduction

THE challenge of the effective delivery of anticancer drugs to solid tumors. High interstitial fluid pressure, dense extracellular matrix, and inhomogeneous perfusion and permeability have impeded the permeation of macromolecules and nanoparticles away from blood capillaries. Extensive chemotherapeutic agents, monoclonal antibodies, or peptides against blood and lymphatic vessels, as well as tumor-penetrating peptides, have been explored to increase the efficacy of drug molecules that enter into tumors. Alternatively, a number of research efforts have been recently devoted to utilizing stimuli--responsive nanoscaled drug carriers to achieve site-specific delivery of drug cargos to solid tumors. Tumor microenvironment (TME) is a promising target for the design of nanocarriers for tumor-targeting drug delivery [1]. The concept of TME-responsive polymeric nanoparticles (PNPs) is established by taking advantages of the relatively higher temperature and acidity, and lower values of glutathione (GSH) concentration and enzyme activity presents in tumor tissues compared to the corresponding values in blood, healthy tissues, and normal cells [1].

Out of various factors of TME, tumor hypoxia is an attractive target because the hypoxic area of solid tumors might eventually become bigger than the corresponding necrotic area because of the rapid proliferation of tumor cells [2]. Furthermore, hypoxia is associated with uncontrolled growth (increasing the risk of tumor

metastasis), and subsequent chemoresistance. The successful delivery of engineered pH-sensitive PNPs to subcutaneously implanted HeLa "hypoxic tumors" in mice via tail vein injection and their significant anticancer efficacy due to the enhanced intracellular drug delivery have been reported by highlighting a new delivery strategy for cancer therapy via targeting tumor hypoxia. Furthermore, a pH-sensitive endosomal escape mechanism that allows for a rapid release of drug molecules in cytosol and for enhanced cytotoxicity of the drug-loaded endocytosed PNPs into HeLa cells has been revealed. The design principle of the biocompatible and biodegradable PNPs, the quality control during the PNP preparation/release experiments, as well as the in vitro and in vivo cellular uptake studies are disclosed [2].

2 Background on Hypoxic Tumors

Inadequate oxygen supply causes hypoxic tumor microenvironments, thereby leading to a range of physiological effects. Briefly discussing the effects of tumor hypoxia, bioenergetic and biosynthetic adaptation, as well as metabolic reprogramming in the tumor microenvironment and hypoxic tumors is warranted [3].

There is a conceptual contradiction between the Warburg effect and the apparent lack of malignancy of many hypoxic tumors, as bioenergetic adaptation to increase ATP production under hypoxia is insufficient to monopolize future carbon sources and create more cancer. The concept of biosynthetic adaptation to maintain redox environment homeostasis and provide better cell survival and broader growth, metastasis, and anticancer drug resistance is thus presented. Effects of hypoxia on various biosynthetic processes that are known to contribute to oncogenesis, tumor evolution, and drug resistance are summarized. Basic biosynthetic pathways derived from glucose, glutamine, and serine in sufficient oxygen conditions, including glycolysis, tricarboxylic acid (TCA) cycle activity, phospholipid biosynthesis, and glutathione synthesis of hypoxic tumors are critical [4].

Additionally, the re-examination of effects of hypoxia and hypocapnia on fallacies of apoptosis and necrosis in cell death as well as on reductive stress and lipotoxicity in aberrant growth is presented. Hypoxic tumors thus exert profound effects on almost all tumor related biological aspects through tumor intrinsic effects and/or the transforming effects of tumor microenvironment on non-tumor cells, as shown in Figure 1. Such multidisciplinary issues are highly appealing, timely, and ripe in basic cancer and research, as well as for conceptual cancer treatment in hypoxic eruption to synergize with anti-angiogenesis, anti-targeted therapy, and other approaches for more effective anti-cancer treatment [5, 6].

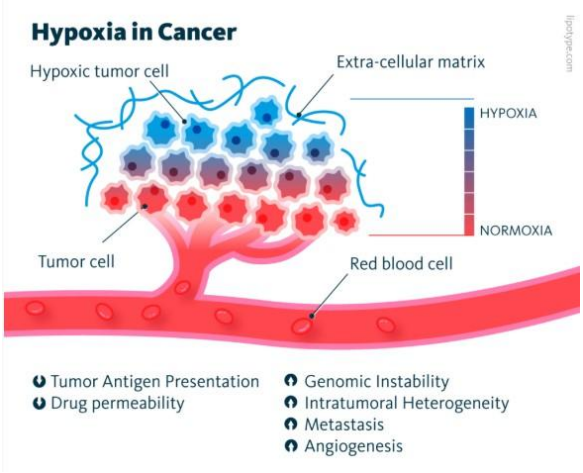

Fig. 1. Hypoxia in cancer [4].

3 Overview of Anticancer Drug Delivery Systems

Various Approaches of Anticancer Drug Delivery Systems There is a growing body of evidence supporting the use of various approaches for the anticancer drug delivery systems. Biomaterials and natural polymers such as polysaccharides and proteins have been employed for the physical and/or chemical encapsulation of drug molecules, allowing the modulation of multiple physiochemical and biological characteristics of polymeric nanoparticles. Therefore, the use of TOMs or natural macromolecules for the design of smart nanosized drug delivery systems seem to be a promising area of research because it will improve the therapeutic index of drug formulations, limit drug resistance, and reduce side effects [7]. Other synthetic approaches utilizing cyclodextrins, dendrimers, and dendritic polymeric nanoparticles have also been employed to modify physiochemical characteristics of drug formulations for improved drug delivery to the tumor sites. Stimuli-responsive drug delivery based on the polymeric carriers can be applied for a controlled release of payloads responding to environmental changes. Among various stimuli-responsive drug-delivery systems, the pH-sensitive ones are the most intensively investigated. The most important strategy for pH-sensitive drug release is to use acid-labile linkers to conjugate drugs covalently to carrier molecules, thus forming prodrugs that are inactive until the linker is hydrolyzed. Most current approaches employ poly (ethylene glycol)-b-poly (amino acid) that provides a highly maneuverable system to develop the anticancer drug delivery systems for tumor-targeted treatment. The presence of acid-sensitive spacers between the polymer and drug promotes drug release in acidic extracellular fluids or after endocytosis in tumor cells [8]. Another strategy is the use of polyelectrolytes with an ionizable group that changes the conformation of polymeric carriers for efficient drug release by altering the environmental pH. Usually, the swapped peptide or polypeptide that can selectively detect pH change from 7.4 to 6.5 in tumor microenvironment has

been incorporated into such polymers that show pH sensitivity. In addition, a pH-sensitive polymeric NP that allows for highly selective anticancer treatment has also been prepared by a stepwise introduction of PEI to D, L-α-tocopherol succinate-dextran polymer, resulting in an insoluble superstructure in physiological buffer solution. Various self-assembled polymeric structures have been developed for the controlled drug delivery, particularly micelles [9]. Such polymeric micelles have a unique lipid-core shape, providing more storage space for the encapsulation of a hydrophobic drug than conventional micelles prepared by amphiphilic block copolymers. This nanocarrier showed good biodegradability and biocompatibility after modification with dextran through hydrophilic chains [10]. On the basis of this system, the classic anticancer drug, DOX, was loaded into micelles of a frameless object that were utilized for triggered drug release under acidic pH condition in extracellular tumor microenvironments. The pH of tumors is effective for tumor targeting. Drug release from pH-sensitive micelles could reduce systemic toxicity and enhance anticancer activity of the delivery system, but it does not solve the problems of mechanism. Historical aspects of the drugs, their interaction with different membrane receptors, and drug metabolism in tumor cells versus those in the normal tissues should also be studied. Ideally, antitumor drugs should rapidly release from the micelles in the acidic microenvironment of endosomes/lysosomes [11], as shown in Figure 2.

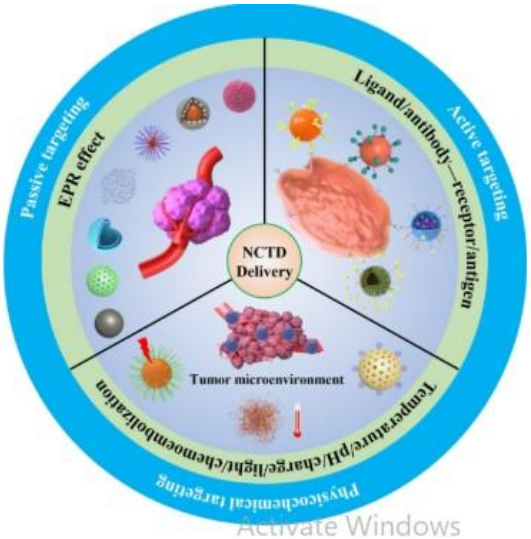

Fig. 2. Schematic diagram of the classification of norcantharidin targeted drug delivery systems [12].

4 pH-Sensitivity in Drug Delivery

Cancer cells outgrowth is accompanied by several physiological changes, including a highly acidic pH microenvironment. In solid tumors, hypoxia becomes more robust as the tumor expands beyond the supply limit of oxygen through nearby blood vessels. Hypoxic tumor cells upregulate the expression of the hypoxia-inducible factors (HIFs) to transactivate the expression of more than 100 downstream responsive genes that are involved in adaptive cellular processes, such as angiogenesis, metabolic alteration, epithelial-mesenchymal transition (EMT), and the inhibition of cell apoptosis. Hypoxia is also linked to the treatment resistance of anticancer therapies and unfortunately usually results in poor prognosis in cancer patients. With a pKa of around 6.5 in physiological saline, camptothecin (CPT) can be released rapidly at acidic pH values, together with steps that prevent metal ions from entering into the polymer backbone to form nanocarriers. pH-sensitive polybutyl methacrylate (PBM) polymer, biodegradable poly(ester-amine) (PEA) copolymers, and more conjugated pH-sensitive moieties have been explored to construct hybrid core-shell pH-sensitive nanocarriers for drug delivery [12].

In response to tumor pH, biocompatible and degradable pH-sensitive star-like poly(N-(5-carboxypentyl) aspartic acid) copolymers have been developed to encapsulate anti-cancer drugs, such as DTX and CPT [2]. Although targeted pH-responsive strategies are being used to increase the accumulation of therapeutics at target sites, the majority of low-molecular weight anticancer drugs are too hydrophilic and cannot be loaded into NPs for delivery. In response to pH reduction, ordered mesoporous silica nanoparticles are generated to continuously deliver anticancer drugs for 20 d and destroy large solid tumors. This work inspired researchers to engineer pH-sensitive drug delivery systems to improve their feasibility and effectiveness. Co-delivery of pH-sensitive genes or RNAs and small anticancer drugs is required to effectively suppress HIFs. Nanotheranostics can be a promising alternative, in which pH/MPA dual-sensitive NPs are synthesized and explored for the controlled release of DTX to dramatically improve therapeutic efficacy against HIF-1α-overexpressing breast cancer [13, 14].

5 Mechanisms of pH-Responsive Release

With the rapid development of tumor detection methods, there are still many challenges in precise tumor treatment with tumor-targeted anticancer drugs. One of the most critical issues is the accurate and controlled delivery of drug to heterogeneous solid tumors. As pH of extracellular or microenvironment of tumor is lower than that of normal tissue, pH-sensitive tumor-targeting drug delivery system is expected to improve the accuracy and efficiency of anticancer drugs, which may enhance the tumor therapeutic effect while hold back the side effect of the drugs on normal tissue. Since some anticancer drugs can induce drug resistance when they are inserted in the blood circulation, polymeric nanoparticles including pH-sensitive and hydrophobic polymer will serve as a carrier to load and stabilize pH-sensitive and hydrophobic anticancer drug at physiological pH level and its solubility and biological activity can be restored at acidic medium due to

protonation and dissociation of pH-sensitive polymer [15, 16]. Most of the studies in application of pH-tumor targeting drug delivery focused on hyaluronic acid, chitosan, and polyacrylic acid polymers, while researches on pH-tumor targeting drug delivery using pH-sensitive and amphiphilic polyacrylic acid methacrylates-based amphiphilic copolymers was scarce [17]. The double-pH-sensitive hydrogel nanoparticles were synthesized using a one-pot method to expect that they have dual buffering and swelling capacity. These two characteristics of the hydrogel nanoparticles is important for drug delivery application, since drug loading and release rates are closely related to buffering capacity of hydrophilic pH-sensitive polymer and drug release rate is related to porous structure formed through swelling induced poly fragmentation of pH-sensitive polymer. This porous structure could be collapsed when nanoparticles are blended with 25, 50, 75 and 100 mol% of cross-linker as an inactive polymer. As there is still no report on application of double pH-sensitive ABA triblock copolymers in drug delivery, it is another vital key point to see a model anticancer drug was loaded into the double-pH-sensitive hydrogel nanoparticles and the amphiphilic hydrogel nanoparticles could be used as a drug carrier of pH-sensitive and hydrophobic diblock copolymers that might be protein resistant and reduce the side effect of drug on normal vascular tissue [2, 18, 19], as shown in Figure 3.

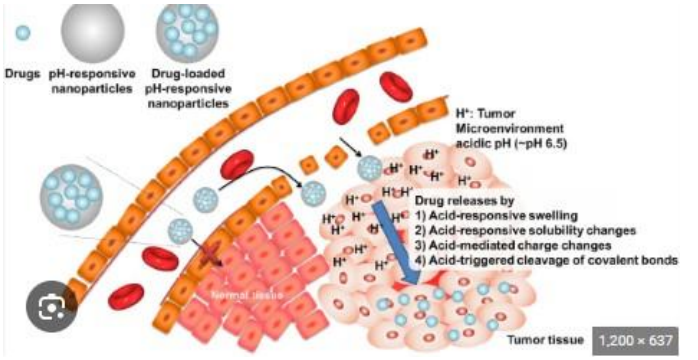

Fig. 3. Mechanisms of pH-responsive release in drug delivery system [20].

6 Advantages of pH-Sensitive Systems

After mitochondrial impairment (low ΔΨm and ROS increase), enhanced levels of pH response (pH ≤ 6.5) and elevated levels of hypoxia (pH 7.6) occur in the cytoplasmic and endosomal/lysosomal levels respectively, which can significantly increase the cellular uptake of nanoparticles based on the pH-sensitive and hypoxia-responsive strategy. By altering the CS and ALD molar ratios, the anticancer drug delivery nanocarriers using a biodegradable hyperbranched polymer platform can be prepared. Upon the simultaneous treatment with photo-irradiation and NIR-I and II light, improved anticancer drug release in the range of mildly acidic and neutral pH was successfully demonstrated. The endocytosis process of the drug carrying nanoparticles based on pH-sensitive polymers can be controlled to achieve a ternary pH-responsive behavior. They can be engulfed through membrane paracellular transportation under the acidic (pH ~6.5) and basic (pH>7.5) environments, which is difficult to invade at pH ~7.0. The possible therapeutic window with high targeted efficiency and low toxicity is expected in cancer therapy by spatiotemporally changing the pH values [20]

Hypoxic tumors are enumerated as one of the most common phenomena which hinder the performance of conventional antitumor therapy. The hypoxia-triggered intracellular drug delivery nanoparticles can not only bypass the severe blood supply dysfunction and overcome the drug resistance problem due to the hypoxia-responsive strategy but also achieve the programmed intracellular release of anticancer drugs through mitochondrial stimuli. The concentration of H2S in the tumor area can be enhanced under the combination of hypoxic microenvironments and continued glycolysis metabolism, which can be used to stimulate the intracellular drug release. The anticancer agent can be efficiently released from the H2S-sensitive polymer platform, as verified through confocal laser scanning microscopy and flow cytometry. The cell viability against HeLa cells can be reduced with the H2S-triggered and controlled drug delivery. The proposed strategy affords an intrinsic supply of nutrients while providing a smart therapeutic nanoplatform for hypoxic tumors treatment, hence extending multifold biomedicinal applications of nanoparticles [21].

7 pH-Responsive Behavior

To evaluate pH-responsiveness, the hydrodynamic radius (HR) of the micelles was investigated. The pH-determined HR of CHA-TPP/C6F5-2c nanocarrier dispersion with water was observed at pH 7.4 due to intermolecular aggregates being formed by the TPP-conjugated biopolymer, which is consistent with that at constant pH 8.4. The average HR was decreased when the pH or ionic strength was decreased below the tested pKa (pH 7.4-7.0, 15mM). This decrease in HR changed from about 300 to about 100 nm due to the dissociation of poly (glutamic acid) moieties from biopolymer chains, leading to the decomplexation of biopolymer-chitosan networks 6. The micelles were checked for pH-responsiveness by changing solutions of pH 8.4 to 7.4, 7.0, and 6.5. The size in HR of the nanocarriers dispersed in water switched to 290 nm at pH 7.4 due to the formation of intermolecular aggregates by acceptor TPP. The average HR was decreased to 160 nm (pH 7.0) and 113 nm (pH 6.5) due to the dissociation of poly(glutamic acid) moieties from biopolymer chains [22].

To further investigate the pH-responsiveness of CHA-TPP/C6F5-2c nanocarriers, GM/DA copolymer block

additional polymer-coated NPs with quencher F-5 or a fluorescein probe were prepared to evaluate Q642-F5-label releasing from CHA-TPP with macromolecules. The release profile of Q646-F5-label from Q646-F5/DA-rPc showed low spontaneous release (<5%) at pH 8.4, 7.4, and 7.0 in 0.1 M phosphate buffer solutions, while the addition of pH 6.5 or 0.05 gentamicin led to a rapid release (up to 80% at pH 6.5, 8 h) due to the recovery of free poly (glutamic acid) (including the probe) above the pKa of the copolymer. Quantification analysis for Q646-F5-label releasing from GBNP showed >80% CM-QQ646-F5 release (cell polymers average size <50 nm) highly within 2 h displaying pH-responsiveness, but pH-insensitivity of CNP (CHA-TPP) with a rapid accumulation of very large enhancing further cellular uptake. To confirm the pH-responsiveness, the cytotoxicity of C6F5-2c nanocarriers to MG63 cells was verified by MTT assay [2]. The cytotoxicity of the C6F5-2c let-VZVG-125NHC approached that of DoX did; that of C6F5-2c scrambled let-VZVG-125NHC was comparable to that of blank CNP without MCP [23].

8 In Vitro Studies on Drug Release

The efficacy of anticancer drugs is limited by poor selectivity of the drug delivery system toward cancer cells due to the similar physiological characteristics of normal and tumor cells. However, the efficacy of drug delivery can be enhanced by exploiting unique properties of tumor cells. The pH of tissues is one of the critical factors that can be leveraged to enhance drug selectivity [24]. The pH of blood and normal tissues is 7.4, whereas hypoxic tumors are more acidic with mildly hypoxic regions having pH 6.5-6.9 and severely hypoxic regions having further reduced pH of 6.0 or lower. In addition, the use of pH-sensitive covalent bonding or association enables controlled drug release with the trigger for release being pH destruction of covalent bond. A number of pH-sensitive drug delivery systems have been reported, including pH-sensitive polymeric nanoparticles (PNPs), micelles, liposomes, gel and dendrimers. [24].

In vitro experiments to study the release of A531 via pH stimulus have been carried out using pH 7.4 PBS and pH 7.4 PBS containing 200 µg/ml of papain. The release of A531 from these systems was measured at 37°C and monitored by fluorescence spectroscopy. In the absence of papain, the release of A531 was almost negligible at both physiological and tumor pH levels. At pH 5.5, the cumulative release level reached above 76.1% after 30 h. In the presence of papain, the system turned to be incapable of retaining A531 at 37°C for more than four hours irrespective of the values of PBS pH. The study indicates the destruction of the pH-sensitive polymeric structure. Results of the study demonstrate that the PNPs employed can effectively retain the anticancer drug A531 at physiological pH and tumor pH levels for prolonged time as well as deliver the drug intracellularly with high efficiency and selectivity upon targeting hypoxic tumors [25].

9 Experimental Design

The designed pH-sensitive polymeric nanoparticles (NPs) were synthesized by conjugating poly (ethylene glyco) (PEG) methyl ether and 4-hexylbenzoic acid (4-HBA) through pH-sensitive phenyl boronate ester bonds. The pH-sensitive characteristics of the obtained NPs were determined using UV-visible spectrophotometer. At pH 7.4, the NPs displayed good stability. When the pH decreased to 6.0, the NPs exhibited a significant increase in absorbance peak at 330 nm, indicating the cleavage of boronate ester bonds and the release of 4-HBA [26].

The anti-cancer drug DOX was chosen as model drug to load into NPs for delivery. The drug loading number of NPs was characterized by adding an equivalent amount of DOX to NPs solution for further investigation, and loading efficiency of DOX was over 60%. The cytotoxicity of NPs was tested on 4T1 cells at different concentrations by adding NPs suspension and incubating for 24 h. The results demonstrated that NPs exhibited low cytotoxicity. They had little effect on cell proliferation and cell death when the NPs concentration was lower than 300 µg/mL. Then, the cellular uptake of NPs by 4T1 cells at various pH values was evaluated. At pH 7.4, the fluorescence intensity of 4T1 cells indicated very low cellular uptake of NPs. At pH 6.5, cellular uptake of NPs correspondingly increased, and pH-sensitivity of NPs could promote cellular uptake and increase accumulation of NPs inside tumor cells [27].

The cellular uptake of DOX-NPs was quantitatively evaluated using the fluorescence intensity of DOX in 4T1 cells treated with DOX-NPs at various pH. DOX-NPs exhibited the same pH-dependent cellular uptake and increased accumulation of DOX in 4T1 cells at decreasing pH. Therefore, the designed pH-sensitive NPs were able to enhance cellular uptake of anticancer drugs for delivery in acidic tumor microenvironment, which might be useful to improve antitumor effect and reduce systemic toxicity of chemotherapeutic agents for cancer therapy. pH-sensitive NPs promisingly improved malignant cancer therapy. However, it was a great challenge for engineering NPs to develop efficient drug delivery system [2]. This study designed novel pH-sensitive polymeric NPs for enhanced intracellular delivery of anticancer drugs in acidic tumor microenvironment [28].

10 Cellular Uptake Studies

The following studies on cellular uptake quantify the cellular uptake of polymeric NPs and their cellular uptake mechanism using various endocytosis inhibitors and pH-sensitive polymer statistics. The results imply endosomal escape. First, the cellular uptake showed significantly higher cellular uptake capability than smaller PS NPs using 6.25 µg/mL NPs at equal mass dose. The attachment and

cellular uptake increased with incubation time and decreased with the presence of free and blank NPs [29]. Colloidal stability experiments confirmed that NPs are dispersed in a physiological range around pH 7 but aggregate below 6, leading to an indicator for pH-sensitive cellular uptake. Cells were treated with a specific chemical prior to the addition of NPs, the mechanisms to inhibit NPs uptake were confirmed, the use of cellular uptake inhibitors on this bioimaging system was justified, and overall pH sensitivity was tested for its efficiency across different tissues. As a model system, a pH-sensitive polymer was synthesized, and NP systems were prepared and characterized. After validation of the bioimaging characteristics, the mechanisms of cellular uptake were studied using a variety of cellular assays; low-ease, multi-angle luminescent NPs were tested as drug-delivery vehicles for in vitro application study [30]. NPs make it possible to enrich a pulsar to achieve cell specificity within a tumor tissue without affecting the surrounding normal tissue and to create a pulsar that allows post-modification of the NPs for adding new functions. In addition to drug loading and delivery, the possibility of using these NPs as customizable biological carriers is also discussed. Using a sequential polymerization strategy, many similar and yet more complex NP formulations for other applications could be easily developed, such as the construction of silica NPs for probing tissue hydration during tumor progression changes. Efficacious delivery of anticancer drugs to intracellular targets remains a critical challenge for cancer therapy. Hypoxia-induced obstacles limit traditional drug delivery systems to hypoxic tumors. pH-sensitive polymeric nanoparticles are engineered as an efficient drug delivery system with enhanced stability in the circulation and intracellular pH-activated drug release. But the cellular uptake mechanism of pH-sensitive PNPs in response to pH varies at different endosomal pH has not been systematically elucidated. In this study, a series of pH-sensitive PNPs is fabricated to investigate effects of feed monomer ratios, polymer molecular weights, and azide content on cellular uptake and endosomal escape efficiency in different pH conditions. PNPs with higher feed carboxylate monomers or lower azide contents exhibit improved cellular uptake and endosomal escape efficiency in mildly acidic conditions owing to their lipid uncoating and assisted membrane fusion. pH-sensitive properties of PNPs are confirmed by cellular uptake and drug release studies under six breast-tumor tissue pH conditions. Importantly, this study provides insights into understanding cellular uptake mechanisms of pH-sensitive delivery systems in response to pH, facilitating design of therapeutic nanocarriers for enhanced therapeutic efficacy in hypoxic tumor microenvironments through improved uptake and endosomal escape in mild acidic environments [31].

11 In Vivo Studies and Efficacy

Aiming for a deeper understanding of the in vivo behaviour and anticancer efficacy of pH-sensitive nanoparticles, which were previously prepared and characterized, preliminary toxicity studies were carried out to determine a suitable drug-free formulation for later efficacy trials. The method was optimized to generate large vesicles (>800 nm) with a narrow size distribution in a scale-up-like process. Doxorubicin was loaded at relatively high amounts. If particles are stable in terms of size for more than 7 days, a burst release is seen in simulated blood fluid, which is partly ionizable. Since no toxicity was observed, NPs were injected intravenously into xenografted mice for tracing their fate in tumours using MNPs. It was shown that particles can accumulate in tumours versus normal tissue over time. The on-demand release of the drug was shown at acidic pH in vitro. To prove the efficacy of applying NP vehicles, early trials were carried out comparing the drug-free carrier with the conjugated and free drug. The tested formulation, which was optimized for dosing and schedule, led to a significant reduction in tumour growth versus the control animals [32]. Despite the large number of polymeric nanodelivery systems, there is still room for improvement in therapeutic efficiency. Most reported nanodevices for controlled release are based on drug encapsulation, which can lead to undesired drug leakage and an increase in systemic toxicity. To overcome this drawback, a strategy for covalent drug conjugation to the nanodevice was applied. An effective therapeutic polymeric PEGylated nanosystem for controlled pH-sensitive drug release was characterized and evaluated on breast cancer and lung cancer cell lines. A significant reduction in the required drug dose to reach its half maximal inhibitory concentration was achieved by conjugation of the drug to the nanoparticles, leading to an improvement in the therapeutic index. The genotoxic effect of this nanodevice in cancer cells was confirmed by nucleus histone H2AX specific immunostaining. A pH-responsive therapeutic polymeric nanodevice was characterised and validated in vitro for controlled anticancer drug release [33].

12 Challenges in Nanoparticle Delivery Systems

Nanoparticles (NPs) have the potential to improve drug bioavailability while minimizing toxicity in healthy tissues, thereby enhancing anticancer efficacy. They can reduce drug side effects and achieve time-dependent drug release. However, successful NP delivery systems that enter the drug candidate for anticancer therapy need to circumvent a series of biological barriers from injection site to delivery site. There are three major barriers against NP delivery systems: diffusion barriers, filtration barriers, and cellular uptake barriers. The profound awareness and understanding of these barriers can significantly enhance

the efficacy of NP drug formulations [34].

There are two major diffusion barriers against NP delivery systems. NP drug formulations injected into blood circulation must diffuse out of capillaries and reach tumor tissues to exert their efficacy. Tumors can be uniquely associated with leaky blood vessels and defective lymphatic drainage. However, this passive targeting, which primarily relies on the abnormal vasculature for preferential accumulation of NPs within tumors, may be inefficient because of the consideration of particle size. It is often believed that small NPs (5 to 20 nm) diffuse rapidly into tissues and are rapidly eliminated by kidneys, while larger NPs (>200 nm) usually have a prolonged circulation time, but a diminished tumor accumulation due to the decreased EPR effect. Consequently, it has been urged to develop an early-stage NP formulation that enables high tumor accumulation within the period of optimal blood half-life. Since hypoxia exists in all solid tumors, another potential approach to overcome the diffusion barrier is the endowment of tumor-activated NP delivery systems responding to the hypoxic microenvironment [35].

Once the NPs diffuse out of blood circulation, they encounter filtration barriers. The reticuloendothelial system (RES), consisting of macrophages in constitutional organs, acts as a critical line of defense in host immune response to recognize and eliminate the foreign agents from blood circulation. In vivo bio-distribution results have indeed supported that a large number of NPs accumulate in the RES organs. To avoid RES recognition, stealth NPs, such as PEGylated and zwitterionic NPs, have been extensively designed and fabricated. The suppression of protein adsorption on PEGylated NPs is prior to enhance circulation stability and reduce uptake by RES organs. However, it was found that once the protein was adsorbed onto the nanoparticle surface, the lipopolysaccharide layer became a new recognition marker by RES cells. This results in that many PEGylated NPs lack long circulation time and passive targeting to tumors. Furthermore, studies have demonstrated that the biodegradability of stealth polymer chains plays a key role in determining NP dynamic alterations in blood circulation. Alternatively, transient stealth NPs or zeta potential switching NPs are promising to enhance blood circulation and bio-distribution in the RES organs for drug delivery in vivo. It is noteworthy that the following toxicity and degrading products of the polymers need to be fully evaluated [36]

Permeability and retention effects of tumor vasculature are the principles for passive targeting of NP formulations into tumor treatment. However, there are three challenges regarding this phenomenon. First, the abnormal structure of tumor blood vessels leads to the EPR effect, which is often over-rationalized. Experimental models behind the good permeability of tumor vasculature, such as xenograft and transgenic models, may not mimic actual tumor vascularity. For instance, the rodent bearing rapid tumor growth forms loose, saccule-structured vessels with large pore sizes in minutes. In contrast, tumor in humans grows slower than rats for many years. Therefore, the tumor vascular structure in humans is much tighter, leading to a disappointed EPR effect. Moreover, the experimental models with fast growing tumors are often associated with angiogenesis and increased permeability, while robustly established tumors are vascularized with mature, more quiescent, and less permeable structures. There are few successful examples of passive targeting to solid tumors even in preclinical models. Thus, it is urgent to enhance active targeting to tumors by using ligand-mediated or exogenous stimulus, taking advantages of both passive and active strategies [37].

13 Conclusion

In the past few decades, nanotechnology has contributed to the development of a wide variety of nanosized carriers for drug delivery. The main advantage of nanocarriers over traditional approaches is the possibility of simultaneously delivering multiple drugs and genes to specific types of cells. On the basis of the evaluation of drug delivery systems, the self-assembly of pH-sensitive polymeric amphiphiles is becoming one of the most powerful skills to prepare nanoparticles or nanomicelles for hydrophobic and hydrophilic drugs. The expectation also focuses on the design and optimization of the composition, structure and formulation routes of the amphiphilic copolymers to control the properties of the nanoparticles. Hypoxia-sensitive nanocarriers for drug delivery, imaging, combination therapy, and the promotion of the anti-cancer immune response have also appeared recently. Targeted treatment of hypoxic tumors has been applied using molecular probes, nanoparticle-fluorophore conjugates, and hypoxia-sensitive drug delivery systems. Recent advances in biomaterial engineering have led to ingenious designs of hypoxia-sensitive macromolecules. The design strategies include the incorporation of various hypoxia-sensitive moieties as cross-linkers, internal components, or ligands and bioconjugation with small-molecule modification or biomacromolecules or biomolecules.

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