Chapter 11
Polymer-Based Tumor-targeted Nanosystems

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Abstract Polymer-based delivery systems for tumor targeting may revolutionize cancer therapeutic strategies by effective delivery of drugs or diagnostic agents by virtue of passive or ligand-based active targeting mechanisms. Thus polymeric carriers will hopefully surmount the drawbacks of conventional cancer therapeutics, such as undesirable biodistribution, drug resistance of cancer cells, and systemic side effects. For this purpose, intense work is underway to develop polymer-based platforms, composed of polymeric nanoparticles (NPs), polymer micelles, polymersomes, polyelectrolyte polyplexes, polymer-lipid hybrid systems, and polymer-drug/protein conjugates as a suitable option for cancer treatment. By versatile polymer chemistry, the conversion of a variety of polymers into functional carriers is possible. Although first polymeric NP systems are already approved for clinical use, the gap between the bench and the bedside remains to be enormous, mainly because of two reasons. Pharmaceutical grade production of macromolecular drug formulations sets challenging demands on precision chemistry, purification, and high-end analytics. Molecular and pharmacological heterogeneity of tumor within a patient, between different patients and between different types of cancer, have resulted in a disparity between preclinical and clinical studies. This review aims to provide a detailed insight in tumor-targeting aspects of various polymer-based nanocarrier systems, to state current trends and introduce novel concepts, presenting examples of drug delivery and bioimaging of cancer.

Keywords Bioimaging · Drug conjugates · Nanomedicine · Polymer micelles · Polyplexes

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11.1 Introduction

At present, numerous polymeric nanosystems are being developed as cancer therapeutics. A broad spectrum of possible chemical modifications facilitates dynamic designs, where tumor-specific pharmacology [1, 2] and microenvironment can modulate improved delivery and release at the tumor site [3, 4]. Applied polymeric systems span the full nanomaterial size scale, from single polymer chains and drug conjugates up to large nanoparticle assemblies [5–12]. They can be classified by their unique physicochemical structures and properties, including solid polymeric NPs, polymeric micelles, polymer conjugates, polymersomes, polyplexes, and polymer-lipid hybrid systems [10–15]. Polymer NPs can be defined by their composition and morphology in a central core and a homogeneously or heterogeneously monolayer or multilayer shell. The therapeutic agent may either be conjugated to the NP, encapsulated between core and shell, or incorporated within the polymeric core. For a schematic overview of polymer-based platforms, see Fig. 11.1.

Polymers employed for fabrication of these nanocarrier platforms may be either of natural origin, such as albumin [16], hyaluronic acid (HA) [17], chitosan (CS) [18], and sodium alginate, or of synthetic origin, such as polyacrylic acid (PAA), polyglycolic acid (PGA), poly(lactide-co-glycolide) (PLGA), polylactic acid (PLA), dendrimers, and hyperbranched polymers [19] (for chemical structures, see Fig. 11.2). The design of polymeric nanosystems can be tailored for transport of a variety of drugs, proteins, nucleic acids, and bioimaging agents.

A prolonged blood circulation time is considered as a critical requirement for a preferred accumulation at the tumor site via the enhanced permeability and retention effect (EPR) [1, 2]. Therefore, nanosystems have to be designed to protect drugs from clearance by the kidney, liver, and reticuloendothelial system from unfavorably

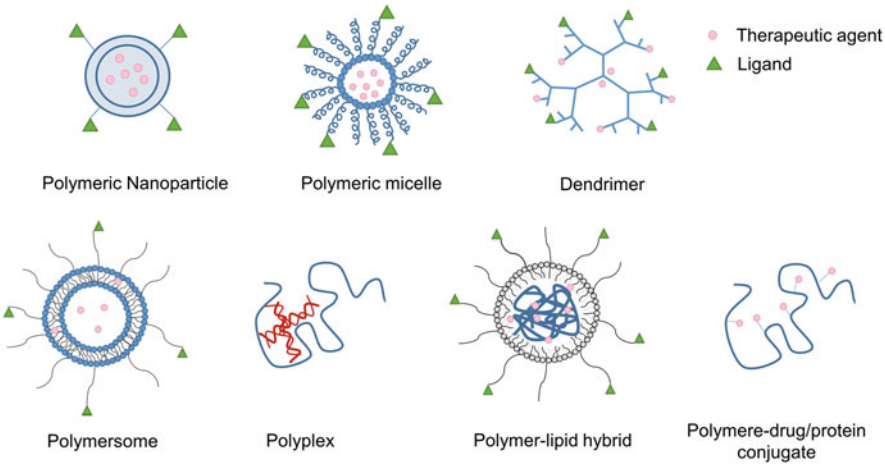

Fig. 11.1 Schematic overview of polymeric nanosystems. The blue color represents the polymeric platform. (Reprinted with permission from Ref. [14]. Copyright © 2019; DOVE Medical Press)

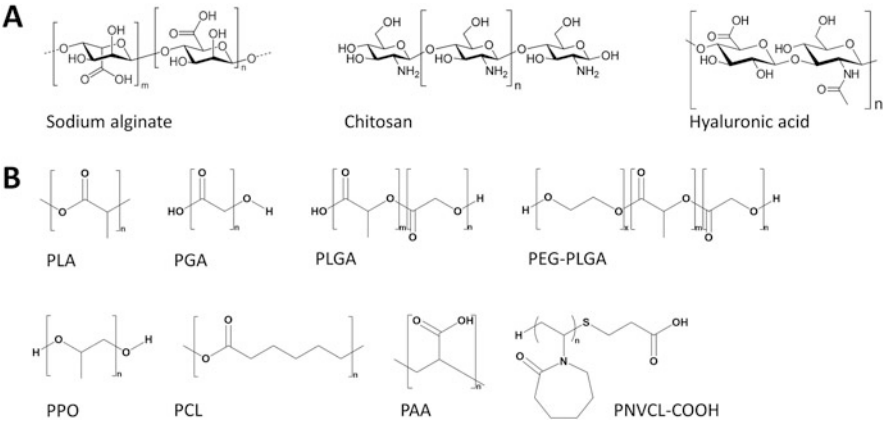

Fig. 11.2 Carrier polymers. (**a**) Natural polymers: sodium alginate, chitosan (CS), hyaluronic acid (HA). (**b**) Synthetic polymers: poly(lactic acid) (PLA), polyglycolic acid (PGA), poly(lactide-co-glycolide)(PLGA), poly(ethylene glycol)-poly(lactide-co-glycolide), (PEG-PLGA), poly(propylene oxide) (PPO), poly-ε-caprolactone (PCL), poly(acrylic acid) (PAA), poly(N-vinylcaprolactam) (pNVCL)

fast metabolism during systemic circulation. For this purpose, functionalization of the polymeric framework with shielding moieties like polyethylene glycol (PEG) [20–22], N-(2-hydroxypropyl) methacrylamide (pHPMA) [23, 24], hydroxyethyl starch (HES) [25], hyaluronic acid (HA) [26], poly(2-oxazoline) [27], or polysarcosine [28] can help to reduce nonspecific distribution [29], to achieve longer blood circulation times, and to reach tumor target tissue. In addition to passive targeting processes, active drug targeting by targeting ligands (peptide, aptamer, antibody, antibody fragment, small molecule) for specific tumor cell surface antigens [14, 30–36] and tumor microenvironment (TME)-triggered programmed drug release and activation mechanisms [3, 4] can be utilized. Polymer-based intracellular drug delivery may also overcome multidrug resistance (MDR) processes. Altogether, such measures should result in an increased antitumor efficacy with minimal systemic side effects [37–40].

However, despite their numerous advantages, polymeric nanosystems also have disadvantages. Depending on the application and the type of drug, various complex synthetic procedures and nanosystem assemblies may have to be applied. Such methods include spontaneous self-assembly by electrostatic and other noncovalent interactions, solvent evaporation, nanoprecipitation, emulsion diffusion, and salting out [41–43]. In some cases the acidity of their degradation products, the large-scale production, and the reproducibility of batch to batch in their synthesis are still a challenge for many polymer-based nanosystems [44]. In addition, there are still many unanswered questions about their toxicity profile and their long-term biological effects. Reviewing published work, Wilhelm et al. highlighted the real efficacy

of only 0.7% of the dose of nanoparticles accumulating at the target tumor site on average [45]. This suggests that there definitely is room for further optimization.

The excellent therapeutic potential at both preclinical and clinical development stages led to diverse applications and perspectives of polymer-based nanosystems in bioimaging and nanomedicine. The fact that some formulations are already in clinical use further validates the efficiency of polymeric platforms for delivery of anticancer agents. This review details the tumor-targeting aspects of various polymer-based nanocarrier systems, focusing on polymeric nanoparticles, micelles, polymersomes, polyplexes, and polymer conjugates, using examples from recent research related to this field.

11.2 Polymeric Nanoparticles (NPs)

Polymeric NPs are solid colloidal systems in which the therapeutic agent is encapsulated, entrapped, adsorbed onto the polymeric matrix, or dissolved. Nanoparticles can be subdivided into different categories depending on their process of formation. The structure of the arising polymeric NPs may differ from nanocapsules (vesicular reservoir systems which are formed in a core-shell arrangement, in which the drug is entrapped in oily or aqueous cavity surrounded by a polymer membrane) to nanospheres (matrix systems in which the active substance is dispersed in the particles) [46–48].

The polymers used to create NPs are mainly biodegradable polyester, which are very suitable for biological systems [49]. Typical polymeric systems for passive and ligand-targeted delivery of therapeutics are poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA) (approved by the FDA for its biodistribution, biocompatibility, and biodegradability [50, 51]), poly-ε-caprolactone (PCL), and the evolution of PLGA, PEGylated PLGA (PEG-PLGA) as synthetic developed polymers [52]. Furthermore, there are also naturally occurring polymers used in drug delivery such as chitosan (CS), gelatin, albumin, and sodium alginate. However, synthetically polymers have proven to be more pure and reproducible when compared to their natural counterparts, which led to their preference in terms of therapeutic application [53]. Table 11.1 provides an overview of selected recent examples of NPs.

PLGA NPs can be employed for gene therapy purposes and for the delivery of bioactive agents such as proteins or drugs [44, 54]. For this reason, PLGA is one of the most important polymers for the development of anticancer drugs [55]. Chittasupho et al., for example, documented a NP system targeting an immunologically active receptor, intercellular adhesion molecule-1 (ICAM-1, CD54), by conjugating the cyclo-(1,12)-PenITDGEATDSGC (cLABL) peptide to the surface of the NP. As an anticancer drug, doxorubicin (DOX) was delivered. The PLGA-cLABL-DOX-NPs showed more rapid cellular uptake compared to NPs without shell functionalization in an A549 cancer model. The cytotoxicity evaluation of cLABL-targeted NPs compared to free drug showed similar IC50 values, indicating that the activity of DOX released from NPs was retained [56].

Table 11.1 Polymeric nanoparticles developed for delivery of drugs to treat various cancers

Carrier system	Targeting ligand	Drug	Cancer cell line	In vitro and in vivo studies	References
PLGA	cLABL	DOX	Adenocarcinomic human alveolar basal epithelial cells (A549)	In vitro	[56]
	–	DTX, TPGS	Human cervical carcinoma cells (HeLa)	In vitro, in vivo	[57]
	HA	SLM PTX	Breast cancer cell line (MCF-7)	In vitro	[58]
	OX26 mAb	TMZ	Human primary glioblastoma cell line (U215, U87)	In vitro	[59]
PEG-PLGA	A10 aptamer	DTX, PTX	PSMA-overexpressed LNCaP	In vitro, in vivo	[62]
	–	PTX	Human cervical carcinoma cells (HeLA)	In vitro, in vivo	[63]
	ITEM4	–	Glioblastoma (KR158)	In vitro, in vivo	[64, 65]
PCL	HA	CAP	Urethane-induced lung cancer (A549)	In vitro, in vivo	[66]
PEG-PCL	–	PTX TMX	Human ovarian adenocarcinoma cells (SK-OV-3)	In vitro, in vivo	[67]
	PD-L1	DOX	Gastric adenocarcinoma (MKN45 MGC803, HGC27)	In vitro	[68]
Poly (Cys-PCL)	–	PTX	Breast cancer cell line (4 T1)	In vitro, in vivo	[69]
PEG-CS	MTX	MTX, MMC	Hepatocellular carcinoma (H22)	In vitro, in vivo	[70]

Abbrevations:
CAP capsaicin; *cLABL* cyclo-(1,12)-PenITDGEATDSGC; *DOX* doxorubicin; *DTX* docetaxel; *HA* hyaluronic acid; *ITEM4* CD266 (TWEAK receptor) monoclonal antibody; *LNCaP* androgen-sensitive human prostate adenocarcinoma cells; *MMC* mitomycin C; *MTX* methotrexate; *OX26 mAb* CD71 TfR (transferrin R) monoclonal antibody; *PCL* poly-ε-caprolactone; *PD-L1* programmed death-ligand monoclonal antibody; *PEG* poly(ethylene glycol); *PEG-CS* PEGylated chitosan; *PLGA* poly(lactide-co-glycolide); *poly(Cys-PCL)* poly-dimethyl L-cysteine-poly-ε-caprolactone; *PSMA* prostate-specific membrane antigen; *PTX* paclitaxel; *SLM* salinomycin; *TMX* tamoxifen; *TMZ* temozolomide; *TPGS* D-α-tocopheryl polyethylene glycol succinate

Zhu et al. reported a PLGA-based NP system delivering docetaxel (DTX) together with vitamin E, D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), as a co-delivery system for HeLa cells in vivo and in vitro. The results showed that TPGS can act as a pore-forming agent with dual function for the production of porous NPs. This leads to favorable pharmacokinetic properties (higher drug encapsulation efficiency and faster drug release) and an active matrix component that provides (P-gp) ATPase and drug efflux inhibition to overcome the MDR of cancer cells. In sum, the strategy of co-delivery of anticancer drugs with TPGS by PLGA NPs was found as advantageous [57].

A PLGA NP co-delivery system, based on encapsulation of salinomycin (SLM), a drug against cancer stem cells (CSCs), and paclitaxel (PTX), a drug against cancer cells, was developed by the emulsion solvent diffusion method. Hyaluronic acid (HA) was coated onto the surface of the NPs as targeting domain to target CD44 receptors, which are overexpressed on CSCs. The results showed the specificity of simultaneous application of both drugs toward the tested MCF-7 cells. Furthermore, a longer circulation time was achieved by the coating, indicating the improved bioavailability of drugs from this nanoparticulate system [58].

The group of Pereira reported the encapsulation of temozolomide (TMZ) in PLGA NPs, which were functionalized with an OX26 type monoclonal antibody for transferrin receptor (TfR) targeting for treating glioblastoma tumor cells, since the TfR is highly overexpressed in this tumor type. U215 and U87 were used to evaluate the in vitro cytotoxicity of the NPs loaded with TMZ, showing an enhancement in anticancer activity of TMZ in comparison to free TMZ. The functionalization with the monoclonal antibody for TfR proved to be advantageous in enhancing the cellular internalization of the NPs into glioblastoma cells, suggesting a selective uptake mechanism [59].

PEG was incorporated into PLGA NPs with the purpose of providing a functional site for surface conjugation of targeting agents, for improving surface properties, and to overcome the limitation of PLGA to provide a very slowly drug release, due to its high crystallinity and low degradation rate [55, 60]. However, a small drawback to PEGylation was the accumulation of high molecular weight PEG in tissues such as the liver [61]. Cheng et al. presented functionalized PEG-PLGA NPs with A10 aptamer as a ligand against prostate-specific membrane antigen (PSMA)-overexpressing LNCaP cancer cells. The PEG-PLGAA10 aptamer NPs reported a nearly fourfold increased delivery of NPs to tumors as compared to equivalent NPs lacking this targeting ligand [62].

Moreno et al. employed PEG-PLGA NPs as a carrier for PTX to improve its therapeutic index. PTX-loaded NPs on HeLa cells were found to be three times more cytotoxic than taxol. In vivo, PTX-loaded NPs in transplantable liver tumor-bearing mice showed marked tumor growth inhibition and increased survival compared to taxol. This resulted from EPR phenomena with PTX-loaded NPs and their ability to maintain drug levels in the blood for a longer time [63].

The group of Kim designed and characterized a nanoformulation with decreased nonspecific adhesiveness and receptor targeting (DART) based on PEG-PLGA targeted with ITEM4 monoclonal antibody against the fibroblast growth factor-inducible 14 receptor (Fn14), which is upregulated in invasive glioblastoma. Tests were done in vitro (KR158 cells, generated from NF1 and p53 mutant mice) and in vivo (orthotopic murine malignant glioma), resulting in improved brain tissue dispersion and tumor cell uptake. In addition, a longer tumor retention was observed in vivo compared to the non-targeted version of the NPs [64, 65].

Parashar et al. developed a NP system containing PCL with conjugated HA as CD44-targeting unit for the delivery of capsaicin (CAP) against urethane-induced lung cancer. A superior performance of HA-PCL-CAP NPs in terms of A549 cell killing was reported when compared with CAP and PCL-CAP. The therapeutic

in vivo efficacy in urethane-induced lung carcinoma was tested in a rat model, revealing a reduced tumor volume, restoration of oxidative stress marker, and improved animal survival rate, when compared to plain CAP and PCL-CAP NPs without surface functionalization [66].

Biodegradable PEG-PCL NPs loaded with PTX and tamoxifen (TMX) were found to be effective in overcoming multidrug resistance in ovarian adenocarcinoma. Such a NP system could lead to tenfold and twofold lower IC50 cytotoxicity values in SKOV3 cells, respectively, in comparison to free drug solution. Upon intravenous administration of PTX-TMX combination in PEG-PCL NP formulations, significant enhancement in antitumor efficacy and negligible treatment-associated toxicity were observed, as measured by body weight changes, blood cell counts, and hepatotoxicity [67].

Another PEG-PCL NP approach was described by Xu et al. They constructed PEG-PCL NPs with a surface-coated programmed death-ligand (PD-L1) monoclonal antibody for targeting the delivery of DOX as anticancer drug against gastric cancer (GC). The cellular uptake of MKN45 overexpressing PD-L1 showed that the functionalized NPs achieved significantly higher cellular uptake in comparison to non-targeted NPs, which was additionally underlined by the results of the in vitro cytotoxicity experiment (MGC803, MKN45, and HGC27). PEG-PCL-PD-L1-DOX NPs were significantly more toxic than their untargeted counterpart or free aqueous DOX. Furthermore, PEG-PCL-PD-L1-DOX NPs induced cell apoptosis and enhanced G2-M arrest in cancer cells, indicating the inhibition of microtubule synthesis [68].

Zhang et al. generated a novel pH and redox dual-sensitive polymeric NP for anticancer PTX delivery, by using PCL as polymeric main structure and modifying it with poly-dimethyl L-cysteine (Cys) via a pH-responsive imine bond. Poly(Cys–PCL)-PTX NPs released PTX in significant amounts only at mildly acidic pH and high concentration of GSH, but with almost no burst release under physiological conditions of plasma. Poly(Cys–PCL)-PTX were more cytotoxic to 4T1 cancer cells than pure PTX alone, showing efficient delivery to the tumor cells. In addition, in vivo results revealed a strong tumor inhibiting ability, good drug tolerability, and biosafety [69].

Jia et al. processed PEGylated chitosan (CS) NPs for the co-delivery of loaded methotrexate (MTX) and mitomycin C (MMC) as drug delivery system, in which MTX is also employed as a tumor-targeting ligand. The cellular uptake in a HeLa cell model illustrated that MTX modification improved the uptake compared to MMC-PEG-CS NPs, which confirmed to be internalized into the cells by FA receptor-mediated endocytosis. Moreover, MTX + MMC-PEG-CS NPs achieved high accumulation at the tumor site of mice bearing H22 tumors and a more efficient suppression of tumor cell growth than by the delivery of either drug alone, indicating a synergistic effect of the co-delivery [70].

In sum, a series of new polymeric NPs have been recently developed. Conjugation with tumor-specific ligands like antibodies, peptides, small molecules, and vitamins was found as a potent approach to enhance antitumoral efficacy. Polymeric NP formulations are the most progressed research field in polymer-based medicine,

resulting in many clinical trials and already FDA-approved products. Indeed, in 2013, two of the top ten best-selling drugs in the USA were polymeric drugs, Copaxone® (glatiramer acetate, a immunomodulatory currently used to treat multiple sclerosis) and Neulasta®(pegfilgrastim, a stimulator of the production of white blood cells) [71]. A prominent example for cancer treatments is Abraxane® (FDA approved in 2005 for breast cancer, 2012 for non-small-cell lung carcinoma (NSCLC), and 2013 for pancreatic cancer), which consists of nanoparticle albumin-bound PTX. In addition, further clinical trials with Abraxane® are currently underway. Currently, a huge variety of trials is ongoing for cancer treating NP formulations. BIND-014, for example, a PEG-PLGA NP delivering docetaxel for the treatment of metastatic prostate cancer and NSCLC, was evaluated in a clinical phase II trial [72, 73]. Another promising candidate is CT-2103 (Opaxio, Xyotax). This NP is formulated out of poly(L-glutamic acid). It delivers PTX for treating ovarian cancer in a phase III trial. Additionally, CT-2103 is tested in a phase III trial, in combination with gemcitabine, to treat advanced NSCLC [15].Moreover, NKTR-102, a four-arm PEG polymer structure for delivering irinotecan, is under clinical evaluation against metastatic breast cancer and colorectal cancer (in combination with cetuximab) [15].

11.3 Polymeric Micelles

Polymeric micelles are amphiphilic self-assembled nanostructures consisting of a core with hydrophobic properties, which allows the incorporation of lipophilic drugs. Furthermore they possess a hydrophilic exterior surface, to protect the drug in the aqueous environment [74, 75] and for stabilizing the polymeric micelle against in vivo recognition by the reticuloendothelial system. Through such stealth-like properties, a longer circulation time in the bloodstream can be obtained [76, 77]. The size and morphology of the assembled micelles of normally 10–100 nm can be controlled through careful design of the hydrophobic/hydrophilic balance. A proper size in the sub-100 nm range may help the accumulation in cancerous tissue [78]. Other properties would be the reduced nonspecific interactions with biological components because of the biocompatible polymer shell. In addition, polymeric micelles have the capability of controlling drug release and can be structurally modified to improve their characteristics [79]. Because of their high molecular mass, the use of di- or tri-block copolymers has led to lower critical micelle concentration (CMC) guiding to a slower dissociation rate upon dilution, resulting in a higher stability in comparison to surfactant-based micelles (e.g., polyethoxylated castor oil or Cremophor® EL, polysorbate 80 or Tween® 80) [80], which extends blood circulation of nanomicelles. Table 11.2 provides an overview of selected recent examples of polymeric micelles evaluated as antitumoral agents.

Genexol-PM is an approved drug based on the development of polymeric micelle formulation of PTX, which consists of poly(ethylene glycol)-*block*-poly(D,L-lactic

Table 11.2 Polymeric micelles developed for delivery of drugs to treat various cancers

Carrier system	Targeting ligand	Drug	Cancer cell line	In vitro and in vivo studies	References
PEG-b-PLA	–	PTX	Breast, ovarian, lung, and head cancers	Phase I	[82]
	–	(o(LA (8-PTX)	Breast cancer cell line (4 T1)	In vitro, in vivo	[83]
	–	AZD2811	Colon adenocarcinoma (SW620)	In vitro, in vivo	[84]
PLGA		DOX	Human cholangiocarcinoma (HuCC-T1)	In vitro	[85]
PEG-PLGA	FA	DOX	Human nasopharyngeal epidermal carcinoma (KB)	In vitro, in vivo	[86]
	–	Pd(II), Alloc-DOX	Fibrosarcoma (HT1080)	In vivo	[112]
pNP-PEG-pNP-DOPE	GLUT1 scFv	DOX, CUR	Human primary glioblastoma cell line (U87)	In vitro	[87]
PEG-DSPE+ mPEG-DSPE	FA	9-NC	Pancreatic carcinoma, human cervical cancer, human gastric carcinoma cell line	In vitro	[88]
PEG-DSPE	GE11	PTX	Human laryngeal cancer cell line (Hep-2)	In vitro	[89]
PEG-DSPE-PH	PDGF	TMZ	Human primary glioblastoma cell line (U87)	In vitro, in vivo	[90]
H40-P (LG-Hyd-DOX)-b-PEG	cRGD	DOX	Human primary glioblastoma cell line (U87)	In vitro	[91]
PEG-PAC+ PEG-PUC	–	THZ, DOX	Human breast cancer cell line (BT-474, MCF-7)	In vitro, in vivo	[92]
DACHPt-PEG-b-P(Glu)	cRGD	Oxaliplatin	Human primary glioblastoma cell line (U87)	In vitro, in vivo	[93–97]
NOSC	–	PTX	Hepatocellular liver carcinoma (HepG2)	In vitro, in vivo	[103]
p(MDS-co-CES)	–	PTX, herceptin	Human breast cancer cell line (MCF-7, T47D, BT474)	In vitro	[104]
P123-PAE	–	CUR	Human breast cancer cell line, hepatocellular liver carcinoma (MCF-7, HepG2)	In vitro, in vivo	[105]
PEG-pAsp (EDA-DM)	–	PAMAM-Pt(IV)	Lung adenocarcinoma (A549R)	In vitro	[106]
PEG-pAsp (AED)-CA	–	HRP, EIA	Lung adenocarcinoma (A549R)	In vitro	[107]

(continued)

Table 11.2 (continued)

Carrier system	Targeting ligand	Drug	Cancer cell line	In vitro and in vivo studies	References
pNVCL-b-PEG	FA	5-FU	Human endothelial cell line (EA.hy926)	In vitro	[108]
PEG-b-pTyr-LA	cRGD	DOX		In vitro, in vivo	[109, 110]
PEG-PPO-PEG	–	DOX	Adenocarcinoma of the esophagus and gastro-esophageal junctions	Phase II	[111]

Abbreviations:
5-FU 5-fluorouracil; *9-NC* 9-nitro-camptothecin; *AZD2811* Aurora B kinase inhibitor; *cRGD* cyclo (Arg-Gly-Asp-D-Phe-Cys); *CUR* curcumin; *DACHPt-PEG-b-P(Glu)* (1,2diaminocyclohexane) platinum(II)-poly(ethylene glycol)-b-poly(l-glutamic acid); *DOX* doxorubicin; *EIA* ethyl 3-indole acetate; *FA* folic acid; *GE11* EGFR peptide ligand; *GLUT1 scFv* GLUT1 single-chain variable fragment antibody; *H40-P(LG-Hyd-DOX)-b-PEG* H40-poly(L-glutamate-hydrazone-doxorubicin)-b-poly(ethylene glycol); *HRP* horseradish peroxidase; *NOSC* N-octyl-O-sulfate chitosan; *o(LA)* oligo(lactic acid); *P123-PAE* pluronic P123-poly(β-amino ester); *PAMAM-Pt(IV)* platinum(IV)-conjugated cationic poly(amidoamine); *PAsp(AED)-CA* poly (N-(2,2′-dithiobis(ethylamine)) aspartamide)-cholic acid; *Pd(II)* palladium; *PDGF* platelet derived growth factor peptide; *PEG-b-PLA* poly(ethylene glycol)-*block*-poly(D,L-lactic acid); *PEG-b-PTyr-LA* poly(ethylene glycol)-b-poly(L-tyrosine)-lipoic acid; *PEG-DSPE* poly(ethylene glycol)-distearoylphosphatidylethanolamine; *PEG-DSPE + mPEG-DSPE* poly(ethylene glycol) – distearoylphosphatidylethanolamine + methoxy-poly(ethylene glycol)-distearoylphosphatidylethanolamine; *PEG-DSPE-PH* poly(ethylene glycol) distearoylphosphatidylethanolamine-N-palmitoyl-homocysteine; *PEG-PAC + PEG-PUC* poly(ethylene glycol)-poly(carbonate) + poly(ethylene glycol) urea-functionalized poly(carbonate); *PEG-pAsp(EDA-DM)* poly(ethylene glycol)-poly[(*N*′-dimethylmaleoyl-2-aminoethyl)aspartamide]; *PLGA* poly(lactide-co-glycolide); *PEG-PLGA* poly(ethylene glycol)-poly(lactide-co-glycolide); *p(MDS-co-CES)* poly[(N-methyldiethylenaminesebacate)-co-[(cholesteryl oxocarbonylamido ethyl) methyl-bis-(ethylene) ammonium bromide] sebacate]; *pNP-PEG-pNP-DOPE* nitrophenylcarbonyl-PEG3400-nitrophenylcarbonyl-1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; *pNVCL* poly(N-vinylcaprolactam); *PPO* poly(propylene oxide); *PTX* paclitaxel; *THZ* thioridazine; *TMZ* temozolomide

acid) (**PEG-*b*-PLA**) [81]. The increased MTD and lethal dose of 50% (LD50) of Genexol-PM compared to the classic PTX formulation displayed that it was less toxic. Genexol-PM was administered to animals at the same dose as lipid-based PTX; this resulted in the same concentration in the plasma with an increased accumulation of 2–3-fold in the heart, lung, kidney, and spleen. Prominently, Genexol-PM had twice as high PTX levels in tumors [82]. Nevertheless, Genexol-PM is unstable in the blood, is cleared rapidly, and causes dose-limiting toxicity. Therefore Tam et al. synthesized a prodrug for PTX (7-OH), using oligo (lactic acid) as a novel pro-moiety (o(LA)8-PTX) specifically for PEG-b-PLA micelles, gaining higher loading and slower release of o(LA)8-PTX over PTX, showing a better compatibility and stability with PEG-b-PLA in comparison with

PTX-PEG-b-PLA (Genexol-PM) [83]. Another approach in the field of PEG-PLA NP platforms was developed by Asthon et al. They worked with the clinical candidate AZD2811, an Aurora B kinase inhibitor. AZD2811 was successfully encapsulated as organic acid salt. In vitro, a continuous drug release for up to 1 week was observed. A corresponding extended pharmacodynamic reduction of tumor phosphorylated histone H3 levels was achieved in vivo for up to 4 days after a single administration, with a promising accumulation in tumor tissues. This resulted in an improvement in efficacy and tolerability in preclinical models with less frequent dosing [84].

Jeong et al. designed polymeric micelles composed of dextran and PLGA block copolymer for DOX delivery. They used DOX-resistant HuCC-T1 cells as in vitro cancer model. The micelles exhibited approximately fourfold higher cytotoxicity to DOX-resistant HuCC-T1 cells as compared to treatment with free DOX, reflecting the effective uptake of polymeric micelles in the tumor cells for overcoming drug resistance. Free DOX barely gained access into the tumor cells [85].

Yoo and Park took advantage of the folate receptor by conjugating folic acid (FA) onto a DOX-loaded PEG diblock copolymer of PLGA by coupling the ligand covalently through its γ-carboxyl group. The in vitro cytotoxicity study showed an increase in cell uptake and cytotoxicity in a KB cell model. The result for folate-conjugated micelles showed a marked improvement in in vivo antitumor activity compared to non-targeted micelles, with a twofold decrease in tumor growth rate [86].

Sarisozen et al. established a polymeric micelle system, with nitrophenylcarbonyl-PEG3400-nitrophenylcarbonyl (pNP-PEG-pNP) conjugated to 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). The topoisomerase II inhibitor DOX and NF-κB inhibitor curcumin were co-incorporated for treating U87MG cells. In addition, the micelles were decorated with GLUT1 antibody single-chain variable (scFv) as a ligand, which promotes blood-brain barrier transport and glioblastoma targeting. Compared to non-targeted micelles, GLUT1 scFv surface modification increased the association of micelles and the nuclear localization of DOX threefold, which translated into higher cytotoxicity [87].

Folate targeting was also used by Han et al. [88]. They created folate-conjugated polymer micelles composed of a mixture of folate-polyethylene glycol distearoylphosphatidylethanolamine (FA-PEG-DSPE) and methoxy-polyethylene glycol-distearoylphosphatidylethanolamine (PEG-DSPE). Micelles loaded with the anticancer agent 9-nitrocamptothecin (9-NC) were evaluated using a pancreatic carcinoma cell line, a human cervical cancer cell line, and a human gastric carcinoma cell line. These experiments demonstrated a greater ability of the folate-conjugated polymeric micelles to actively attack tumor cells with overexpressed folate cell surface receptors as compared to analogous folate-free micelles or the free anticancer agent [88].

Ren et al. developed PEG-DSPE micelles containing PTX with modification of GE11 as peptide ligand for specific targeting of EGFR overexpressing cancer cell line Hep-2. Hep-2 cell proliferation was significantly inhibited by the GE11-PEG-DSPE-PTX system in comparison to untargeted PEG-DSPE-PTX and free PTX

in vitro. Their results suggest a promising targeting strategy for enhancing PTX's tumor therapeutic effects on EGFR overexpressing cancer cell lines [89]. Likewise, Miller et al. worked with PEG-DSPE. They surface-functionalized the polymer system with a novel PDGF (platelet-derived growth factor) peptide. TMZ was used as cargo. PDGF-micelles encapsulated with TMZ showed specific uptake and increased killing in glial cells compared with untargeted micelles. In vivo studies prove selective accumulation of PDGF-micelles containing TMZ in orthotopic gliomas implanted in mice, and the pH-dependent release of TMZ preferably in the tumor tissue reduced the systemic toxicity [90].

Xiao et al. designed a hyperbranched amphiphilic block copolymer H40-poly (L-glutamate-hydrazone-doxorubicin)-b-poly(ethylene glycol) (i.e., H40-P (LG-Hyd-DOX)-b-PEG) and conjugated it with the peptide ligand (cRGD) cyclo (Arg-Gly-Asp-D-Phe-Cys) for integrin αvβ3 targeting. Unlike the previous approaches, DOX was not encapsulated, but covalently bound onto the hydrophobic segments of the amphiphilic block copolymer. Covalent binding proceeded via acid-labile hydrazone bonds, to enable pH-controlled drug release. H40-DOX-cRGD showed a much higher cellular uptake in U87MG cells due to integrin αvβ3-mediated endocytosis compared to non-targeted micelles, suggesting higher cytotoxicity and a much higher level of tumor accumulation [91].

Ke et al. investigated in their study the potency of cancer stem cell killing with a combination therapy of thioridazine (THZ) and DOX in a polymeric micelle-based system using a mixture of acid-functionalized poly(carbonate) and PEG diblock copolymer (PEG-PAC) and urea-functionalized poly(carbonate) (PUC) and PEG diblock copolymer (PEG-PUC). BT-474 and MCF-7 cells were used as tumor cell culture models. In vivo evaluation was performed using BT-474 xenografts in nude mice. Encapsulated THZ + DOX in the described micellar system showed a significant decrease of cancer volume in comparison to free THZ + DOX in vivo, resulting in a more suppression of effective tumor growth. The combination of co-delivering THZ + DOX achieved stronger antitumor efficacy when compared to single DOX or THZ [92].

Miura et al. developed polymeric micelles incorporating (1,2-diaminocyclohexane)platinum(II) (DACHPt), the parent complex of the potent anticancer drug oxaliplatin, through the metal complex formation-driven self-assembly of poly(ethylene glycol) (PEG)-b-poly(L-glutamic acid) and DACHPt in an aqueous milieu (DACHPt/m). They also used the cyclic peptide cRGD (Arg-Ala-Asp) as a ligand and compared it to cRAD (Arg-Ala-Asp) as a non-targeting ligand control. In contrast to the cRAD micelles, the micelles with cRGD accumulated with a high efficiency in the tumor cell line (U87MG), resulting in significant antitumor effects in an orthotopic mouse model of U87MG glioblastoma [93]. The same system of DACHPt-[PEG-*b*-p(Glu)] is used in several other cancer models by the same research group [94–97]. This type of nanomicelles is under clinical evaluation, for example, against spontaneous pancreatic cancer as a combination therapy with gemcitabine [98, 99], followed by a phase II study against NSCLC, biliary tract, and bladder cancer [100]. Most recently, the group extended their tumor-targeting efforts by incorporation of glucose into platinum-loaded polymeric micelles via the carbon

6 of glucose. By this measure, targeting to GLUT1 receptor which is overexpressed on many tumors as well expressed on vascular endothelium was possible, resulting in enhanced tumor accumulation (both in GLUT1-high OSC-19 and GLUT1-low U87MG tumors) antitumoral efficacy [101]. Analogous GLUT1 targeting into breast cancer stem–like cells was obtained for PLK1siRNA/polymer/gold sub-50 nm nanoparticles, with encouraging antitumoral effects [102].

Jin et al. synthesized an amphiphilic graft copolymer, N-octyl-O-sulfate chitosan (NOSC), for encapsulation of PTX for treating multidrug-resistant cancer. As a cell model, they used HepG2 cells and MDR HepG2 cells (HepG2-P). The micelles had a high cellular uptake, about twofold higher than taxol. A low efflux of PTX triggered optimal cytotoxicity in both cell lines. In a mouse model, the intravenously injected PTX-loaded micelles showed a higher tumor inhibition rate over that of taxol in tumor-bearing mice. This also resulted in longer survival time of mice [103].

Lee et al. reported micellar nanoparticles constructed of a biodegradable and amphiphilic copolymer, poly[(N-methyl bis(ethylene) amine sebacate)-co-[(cholesteryl oxocarbonylamidoethyl) methyl bis(ethylene) ammonium bromide] sebacate], p(MDS-co-CES). The micelles were used as carrier to co-deliver PTX and Herceptin for achieving targeted delivery of PTX to human epidermal growth factor receptor-2 (HER2/neu)-overexpressing human breast cancer cells (MCF7, T47D, and BT474) and an enhancement of cytotoxicity through synergistic activities. The targeting ability of their co-delivery system showed significantly higher cellular uptake in HER2-overexpressing BT474 cells as compared to HER2-negative HEK293 cells [104].

pH-responsive polymeric micelles were also used by the group of Cai et al. They created a pluronic P123-poly(β-amino ester) (P123-PAE). Curcumin served as a drug of choice which was efficiently encapsulated into the P123-PAE micelles. In vitro, curcumin P123 PAE showed similar antitumor activity against MCF-7 and HepG2 cells as compared to solubilized curcumin solution. In vivo, however, a longer circulation time of curcumin P123 PAE was observed, resulting in a longer retention time and therefore a superior pharmacokinetic outcome [105].

Li et al. [106] constructed anionic block copolymers, poly(ethylene glycol)-poly[(*N*′-dimethylmaleoyl-2-aminoethyl)aspartamide] (PEG-pAsp(EDA-DM)) with platinum(IV)-conjugated cationic poly(amidoamine) (PAMAM-Pt(IV)) prodrugs as drug delivery system with appreciable tumor tissue acidity-responsive charge conversion function. The efficient drug penetration together with elevated drug potency contributed to significant growth inhibition of A549R cells. Results demonstrated that this smart polymeric micelle drug delivery system managed to overcome the barriers of tumor tissue penetration and drug resistance. Efficient drug permeation coupled with increased drug potency contributed to a significant inhibition of A549R MCTS growth inhibition. The in vitro results confirmed that overcoming the barriers of tumor tissue penetration and drug resistance is successful with the described dual endogenous stimuli-responsive micelles [106].

Moreover, polymeric micelle systems can also be used as nanoreactors. Li et al., for example, designed one based on ternary block copolymer PEG-pAsp(AED)-CA, consisting of PEG, reduction-sensitive poly(N-(2,2′-dithiobis(ethylamine))

aspartamide) pAsp(AED), and cholic acid (CA) [107]. They applied this polymeric micelle system for co-delivering horseradish peroxidase (HRP) as an enzyme for prodrug activation and ethyl 3-indoleacetate (EIA) as the anticancer prodrug. HRP was integrated into a reduction-sensitive interlayer, whereas EIA was encapsulated in the hydrophobic core of the polymer. Cell survival tests on A549 cells showed high tumor cell killing rates in comparison to unloaded micelles [107].

Prabaharan et al. invented a thermosensitive poly(N-vinylcaprolactam)(pNVCL)-b-PEG block copolymer micelle system conjugated to FA (pNVCL-b-PEG-FA). Self-assembly into stable micelles worked in aqueous solution starting above 30 °C. At the physiological temperature of 37 °C, the release of the encapsulated anticancer agent 5-FU was slow, steady, and therefore more controlled than at a temperature of 25 °C. The 5-FU-loaded pNVCL-b-PEG-FA did not induce remarkable cytotoxicity against EA.hy926 cells but showed cytotoxicity effects against 4 T1 cells, due to the availability of the target cell folate receptor [108].

The group of Zhong generated biodegradable, reduction-responsive polytyrosine micelles (rPTM) based on poly(ethylene glycol)-b-poly(L-tyrosine)-lipoic acid (PEG-b-pTyr-LA). cRGD was used as targeting domain. The created NPs had a high drug loading content of DOX. cRGD-rPTM-Dox NP were tested in vitro and in vivo in an MDA-MB-231 cell line. A prolonged circulation time compared with the non-cross-linked cRGD-PTM-Dox control and significantly better accumulation in tumor tissues as compared to the non-targeted version were reported, outperforming even clinical liposomal DOX-NPs [109]. This NP platform was also successfully tested in vivo against HCT-116 with a nearly three times higher tumor accumulation compared to rPTM-Dox [110].

Juan et al. produced triblock copolymers of PEG and poly(propylene oxide) (PPO) with PEG-PPO-PEG structure for encapsulation of DOX. The so formed drug delivery system was named SP1049C and used in a phase II clinical trial. Patients with inoperable metastatic adenocarcinoma of the esophagus and gastro-esophageal junction, when treated with SP1049C, had high objective response rates and increased median survival, demonstrating superior antitumor activity of SP1049C compared with a standard formulation of DOX [111].

The group of Weissleder recently made use of palladium catalysts to catalyze intracellular reactions by converting prodrugs in their active forms. Palladium can cleave allyloxycarbonyl (alloc) groups under biological conditions. For this purpose, they used PEG-PLGA micelles with Pd(II) encapsulated in the core. The polymeric micelles were intravenously injected in cancerous mice and accumulated in the tumors by passive targeting based on the EPR effect. After 5 h, a prodrug of DOX, protected with an alloc ligand (alloc-DOX) at its active daunosamine amino group, was systemically delivered to the tumors. In the tumor, the accumulated Pd (II)-micelles could catalyze the release of DOX by cleaving off the alloc groups, which lead successfully to tumor regression and apoptosis [112].

In sum, many chemotherapeutic drugs have been formulated into polymeric micelles. Also plenty of polymeric micelle-based systems for tumor-targeting cancer therapy are under current clinical evaluation. Up to now, only Genexol-PM© has been approved, for the treatment of MBC and NSCLC, by the Ministry for Food and

Drug Safety of South Korea. There are ongoing phase III and IV clinical trials for FDA approval [15].

11.4 Polymersomes

Polymersomes are polymeric vesicles, which are fabricated through self-assembly of synthetic amphiphilic block copolymers, consisting of discrete hydrophilic and hydrophobic blocks, in aqueous solutions to form an architecture analogous to liposomes (vesicles derived from phospholipids) [113–115]. Compared to liposomes, polymersomes may have greater stability, storage capacity, and prolonged circulation time [116, 117] and can be easily functionalized by numerous functional groups, e.g., by targeting groups and stimuli-responsive segments [118]. Polymersomes have gained a growing interest in biomedical applications as nanocarrier systems for a variety of payloads. They achieve a programmable drug delivery and increased selective accumulation at tumor sites.

Table 11.3 provides an overview of selected recent examples of polymersomes evaluated in cancer models.

Polyphosphazene-based polymers (PEPs) have been studied as a delivery system of hydrophilic DOX hydrochloride (DOX·HCl) or hydrophobic DOX base (DOX). Xu et al. could show a high encapsulation rate of DOX·HCl and DOX due to strong intermolecular interaction with the PEP polymersomes. In a MCF-7 xenograft model in nude mice, a similar tumor growth inhibition was observed as with free DOX·HCl and DOX. However, a better life span safety and a strong reversal of drug resistance were noted due to improved pharmacokinetics [119].

Another approach of encapsulating DOX in a polymersome system was developed by Upadhyay et al. They used poly(γ-benzyl-L-glutamate)-block-hyaluronan (pBLG(23)-b-HYA(10)) as their polymersome system and evaluated the antitumor efficacy of DOX in an Ehrlich ascites carcinoma (EAC) murine mouse model. Due to the availability of HA on the surface of the polymersomes, CD44 receptor-mediated endocytosis is achieved. This resulted in a prolongation in tumor doubling time and an increased survival of mice [120].

The group of Voit also used DOX as their payload of choice. They developed photo-cross-linking stabilized polymersomes with a pH-adjustable membrane permeability and FA ligands as targeting domain. Poly(ethylene glycol)-block-poly [2-(diethylamino) ethyl methacrylate-stat-2-hydroxy-4-(methacryloyloxy) benzophenone] [PEG-b-p(DEAEMA-stat-BMA)] was used as the building block of their polymersomes. SBC-2 cells (with high FA receptor density on surface) were used for tumor targeting. The treatment of SBC-2 cells with FA-conjugated, Dox-loaded polymersomes arose in higher cell death as compared to SBC-2 cells treated with polymersomes without FA, showing the specific targeting efficiency of the FA modified polymersomes. In addition, when blocking folic receptors of SBC-2 by free FA, the toxicity potential of the polymersome system was significantly reduced, verifying the targeting specificity [121].

Table 11.3 Polymersomes developed for delivery of drugs to treat various cancers

Carrier system	Targeting ligand	Drug	Cancer cell line	In vitro and in vivo studies	References
PEPs	–	DOX	Human breast cancer cell line (MCF-7)	In vitro, in vivo	[119]
PBLG(23)-b-HYA(10)	–	DOX	Ehrlich ascites carcinoma (EAC)	In vivo	[120]
PEG-b-p (DEAEMA-stat-BMA)	FA	DOX	Small-cell lung carcinoma (SBC-2)	In vitro	[121]
PEG-b-cap	PR_b	Cisplatin	Human colon cancer cell line (DLD-1)	In vitro	[122]
PEG-b-P-CPTKMA–co-PEMA	–	Camptothecin	Lung adenocarcinoma (A549), hepatocellular carcinoma (H22)	In vivo	[123, 124]
PEG-Chol-10	–	DOX, 5-FU, LV	Human pancreatic cancer cell line (BxPC-3)	In vitro, in vivo	[127]
POEGMA-PDPA	cRGD	PTX	Peritoneal gastric carcinoma (MKN-45P), colon carcinoma (CT26)	In vitro, in vivo	[129]

Abbreviations:
5-FU 5-fluorouracil; *b-cap* block-poly(γ-methyl-ε-caprolactone); *cRGD* cyclo(Arg-Gly-Asp-D-Phe-Cys); *DOX* doxorubicin; *FA* folic acid; *LV* leucovorin; *PBLG(23)-b-HYA(10)* poly(γ-benzyl-l-glutamate)-block-hyaluronan; *PEG-b-P-CPTKMA–co-PEMA* poly(ethylene glycol)-block-poly-(camptothecin thioketal-linked methacrylate monomer)-co-(2-(pentamethyleneimino) ethyl methacrylate); *PEG-Chol-10* poly(ethylene glycol)-cholesteryl-decyl chain; *PEG-b-P(DEAEMA-stat-BMA)* poly(ethylene glycol)-block-poly[2-(diethylamino) ethyl methacrylate-stat-2-hydroxy-4-(methacryloyloxy) benzophenone]; *PEPs* polyphosphazene-based polymers; *POEGMA-PDPA* poly(oligoethylene glycol methacrylate)-poly(2-(diisopropylamino)ethyl methacrylate P[(OEG) 10MA]20-PDPA90; *PR_b* α(5)β(1) integrin-specific targeting peptide; *PTX* paclitaxel

Kokkoli et al. developed bioresorbable polymersomes for efficient and site-specific delivery of cisplatin to human colon cancer cells (DLD-1) overexpressing α(5)β(1) integrin. As a polymeric basis, PEG-block-poly(γ-methyl-ε-caprolactone) was used. The formed polymersomes were then functionalized with an α(5)β(1) integrin-specific targeting peptide named PR_b, which is highly specific to α(5)β(1) integrin than the conventionally RGD-targeting peptide that on the other hand is capable to bind a broad variety of integrins. Cisplatin-loaded PR_b-functionalized polymersomes displayed enhanced cytotoxicity toward DLD-1 cells compared to untargeted polymersomes. In addition, targeted polymersomes were less toxic to CACO-2 model human epithelial with a low expressed α(5)β(1) integrin rate, verifying the specific targeting of PR_b [122].

Polymersome systems can also be used as nanoreactors, which could be demonstrated by Ke et al. They developed therapeutic nanoreactor with selectively tumor acidity-responsive membrane permeability to activate a cascade reaction to in situ produce hydroxyl radicals (ROS). Their polymersomes are constructed out of

PEG-b-P-(CPTKMA (camptothecin thioketal-linked methacrylate monomer)-co-PEMA (2-(pentamethyleneimino) ethyl methacrylate)) as polymeric basis. Iron oxide nanoparticles were incorporated in the polymer membrane and glucose oxidase into the aqueous core, to trigger the ROS production by Fenton reaction. ROS would then release camptothecin by cleaving the thioketal linker. In an in vivo murine model of A549 and aggressive H22 mouse hepatoma, the nanoreactors proved to own highly effective tumor suppression abilities and at the same time low systemic toxicity [123, 124]. A very similar polymersome nanoreactor based on ROS production, which bursts the vesicle and unloads its cargo, quinone methide for specific tumor killing, was described by Li et al. [125].

Aibani et al. reported a polymersome system consisting of the electroneutral co-polymer constructed with PEG, cholesteryl chains, and decyl chains [126]. This system was used for drug delivery of DOX, 5-FU, and leucovorin (LV). The polymersomes were examined in pancreatic BxPC-3 cells in vitro and in vivo (ectopic BxPC-3 mouse model). The same concentration of the three free drugs served as comparison. In all experiments, the polymersomes had better efficacy and a higher maximum tolerated dose. When injected intratumorally, the tumor volume was reduced significantly as compared to the free drug combination. Furthermore, the polymersomes were less cardiotoxic [127].

Another approach of controlling the stability and cargo release of polymersomes was described by Simón-Gracia et al. They invented pH-sensitive polymersomes for PTX delivery to MKN-45P and CT26 carcinoma in a murine model. The polymersomes were assembled from diblock copolymer poly(oligoethylene glycol methacrylate)-poly(2-(diisopropylamino)ethyl methacrylate, p[(OEG)10MA]20-pDPA90 or pOEGMA–pDPA). The pOEGMA established a stealth-like character against the immune system, and the pDPA block served as a pH sensitizer. The polymersomes were highly stable in aqueous solution at neutral pH for up to 4 months, whereas exposure of pH <6 leads to quick disassembly. The PTX-pOEGMA-PDPA polymersomes showed robust accumulation and penetration in the in vivo tumor model and inhibited the tumor cell growth more efficiently than Abraxane®(albumin-paclitaxel nanoparticles), suggesting more efficient tumor cell uptake and/or cytoplasmic release of PTX [128]. Simón-Gracia et al. also worked on a more specific tumor targeting system in addition to the pH sensibility for their established system by conjugating RGD, for integrin $\alpha v\beta 3$ targeting, onto the polymersome shell. This led to higher tumor-selective accumulation and penetration than untargeted polymersomes, resulting in improved tumor growth inhibition and suppression compared to their other model and Abraxane® [129].

In sum, polymersomes present an encouraging novel alternative to classical liposomal formulations for encapsulation of drugs. For example, DOX-loaded polymeric vesicles made of PEG-block-PCL were able to retard tumor growth in a live animal on a par with the commercially available agent DOXIL® (a clinically administered liposomal formulation of DOX) [116] and therefore may possibly represent an alternative to liposomes like DOXIL® in the future. Furthermore, the polymeric basis of the vesicle membrane provides a broader basis for programmed disassembly than conventional lipid vesicles.

11.5 Polyplexes

Among synthetic nucleic acid carriers, cationic polymer-based nanosystems are widely investigated. An overview of the first five decades in developing nucleic acid – polycation polyelectrolyte complexes termed polyplexes [13] – can be found in references [31, 130]. Polyplexes offer various benefits, such as lower immunogenicity compared to viral vectors and, importantly, the capability of carrying either natural or chemically modified nucleic acid material. However, due to the presence of numerous positive charges on the surface of polyplexes, binding with serum complement proteins and activation of innate immune system, aggregation and nonspecific interaction remain the major issues in their application [29, 131–133]. Consequently, more stable packaging of polyplexes (e.g., by introducing hydrophobic elements or covalent cross-links within the particle core) and shielding the positive surface charges against undesired specific interactions with the bio-macromolecules, such as serum proteins, and avoiding an undesirable immune response are important measures to overcome these problems [134, 135]. These considerations resulted in the design of PEGylated polyplexes, which are polyelectrolyte complexes formulated by the self-assembly of oppositely charged anionic nucleic acids with PEG-polycation block copolymers. Alternatively to the use of diblock polymers, polyplex cores were formed with polycations and subsequently modified with shielding PEG or other shielding polymers [20, 136, 137]. The outer hydrophilic PEG layer of the core-shell architecture provides a stealth effect, minimizing nonspecific interactions and prolonging circulation time in vivo [31, 138]. The plasmid DNA (pDNA) or siRNA is complexed and condensed through electrostatic interactions, hydrogen bonding, coordinative interactions, and hydrophobic interactions [139]. However, entropy-driven electrostatic interactions between the cationic groups of the polymer and the negatively charged nucleic acids are the most prominent way of condensation [13, 31]. Condensation is necessary to prevent degradation and the release of cargo at off-target sites, neutralize negative charges, and reduce the size of larger nucleic acids such as pDNA [140, 141] with the aim of liberating the cargo in the cytosol, in the case of siRNA [142], or the nucleus, in the case of DNA [143, 144]. In both cases, cell uptake by endocytosis into cellular vesicles and endosomal escape mechanisms are involved [145–149]. See Fig. 11.3 for the nucleic acid delivery pathway of polyplexes. Therefore binding to the nucleic acid should be reversible, resulting in a well available therapeutic cargo once the specific target site is reached [150]. Another important factor of influencing the biological activity of polyplexes is the modulation of their shape (folded rod or collapsed sphere) and size, which can be precisely controlled by the length of the polycationic segments of the block copolymers [22, 151, 152] for additionally controlling the packaging of nucleic acid into an appropriate structure to achieve effective gene expression [153].

The most studied cationic polymers for the preparation of polyplex systems have been linear (LPEI) or branched (BPEI) polyethylenimine (PEI) and poly(L-Lysine) (PLL) [31, 154, 155]. PEI displays a high concentration of positively charged amino

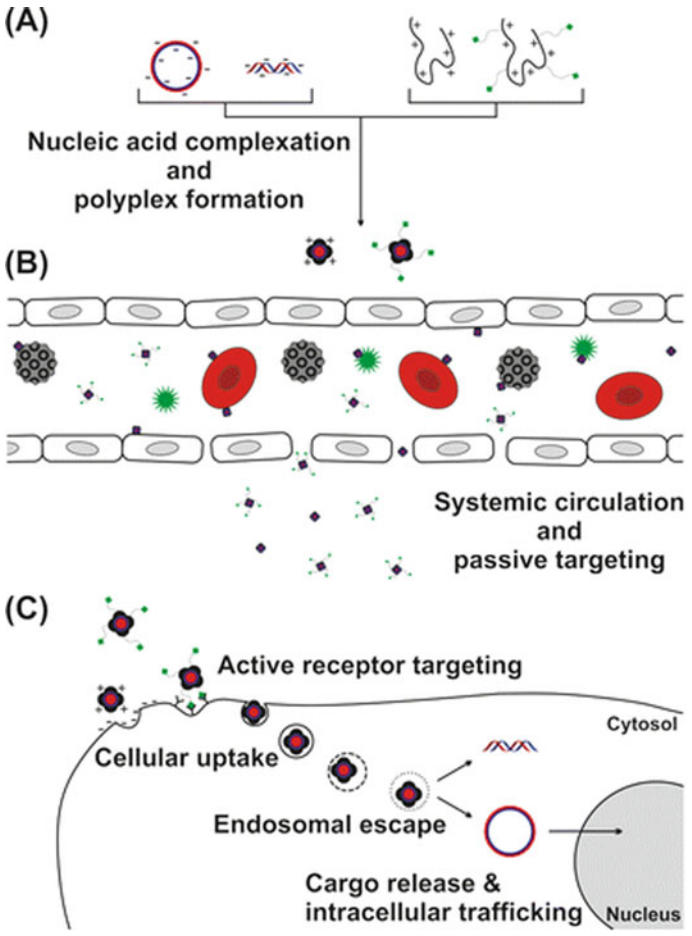

Fig. 11.3 Barriers in the nucleic acid delivery pathway of polyplexes. (**a**) Formation of stable polyplexes, (**b**) avoidance of rapid clearance and unspecific interactions with blood components, and (**c**) cellular barriers. (Reprinted with permission from Ref. [31]. Copyright © 2019; American Chemical Society)

groups (primary to tertiary), which enables effective electrostatic binding and condensation of negatively charged DNA [156]. Additionally, it possesses buffering capacity and polymer swelling at the acidic pH of the endosomes [157]. Optimized versions of PEI have already been applied in human clinical trials for cancer therapy. Nevertheless, PEI has also drawbacks like cytotoxicity and non-degradability. PLL, on the other hand, consists of only primary amines in its side chain, which interact with the DNA for the condensation. However, PLL has no intrinsic buffer-based endosomal escape mechanism, making it inferior as a gene delivery system when compared to PEI [158, 159]. Functionalization with endosomolytic agents such as virus-derived peptides is required to overcome the drawback of polymers such as

PLL [160–163]. Non-degradability of PEI is associated with medium- to long-term cytotoxicity [164]. Therefore the development of biodegradable analogs has been one line of optimization [165–167]. Both PEI and PLL are suitable polymers for polyplexes [168–175] but also pose risks such as triggering the complement activation of the innate immune system, which can lead to anaphylactic shocks [176]. Moreover, their polydisperse chemical nature as well as difficulties in precise modifications hampers their broader development. Therefore, the design of new more defined platforms, such as sequence-defined polymers [177, 178] as carrier systems to tackle these issues, has been a major research focus in pDNA and siRNA delivery. Table 11.4 provides a small selection of recent examples of polyelectrolyte complexes (polyplexes) as antitumoral agents. A wider overview can be found in references [31, 130].

Cationic (oligoethanamino)amide-based polymers (OAAs, see Fig. 11.4) synthesized by using Fmoc-/Boc-protected oligo(ethane amino)acids as building blocks for solid-phase-supported assembly represents one novel promising approach toward fully controlled syntheses of effective gene carriers, as firstly described by [179–184]. In addition to natural amino acids, artificial oligoamino acids (OAAs) such as succinoyltetraethylenepentamine (Stp) were introduced, providing a moderate positive charge density responsible for nucleic acid binding, intracellular endosomal release behavior within tumor cells based on the PEI-like proton sponge effect, and high biocompatibility due to decreased electropositivity and medium-small molecular weights [185]. Dohmen et al. conjugated them with FA for targeted delivery of siRNA in human cervix carcinoma cells. This polyplex system achieved folate receptor-specific cell targeting and silencing of the EG5 gene in receptor-positive tumors. The in vivo administration arose in silencing of the reporter gene and the lack of accumulation in non-target tissues [182].

For optimizing the bioactivity of anOAA polyplex system by folate receptor targeting in a cancer combination therapy, Lächelt et al. used MTX as a dual-functional ligand, inducing a folate receptor-mediated cellular uptake and an intracellular cytotoxic action. Molecularly precise OAAs were synthesized as carriers to encapsulate dsRNA polyinosinic–polycytidylic acid poly(I:C). The bioactivity, concerning dihydrofolate reductase (DHFR) inhibition, cytotoxicity, nucleic acid binding potency, cellular uptake of poly(I:C) polyplexes, and combined antifolate/poly(I:C) toxicity in KB cells, was investigated. More closely, the effect of different degrees of synthetic polyglutamylation on the conjugated MTX ligand was evaluated. The results demonstrated a correlation between polyglutamylation, the efficiency to deliver poly(I:C), and the MTX triggered cytotoxicity. With higher glutamylation degree, the transfection efficiency increased, leading to enhanced DHFR inhibition and higher cytotoxicity [186]. Optimized polyglutamylated MTX was used by Lee et al. [187] as a dual targeting ligand for siRNA delivery. A series of sequence-defined OAAs were prepared for eglin 5 (EG5) siRNA condensation into nanoplexes, targeted with different polyglutamylated MTX ligands. In vivo studies with KB tumor-bearing mice displayed an increase of intratumoral retention (168 h) of the siRNA, when compared to non-targeted control polyplexes (48 h). For this combination of MTX-conjugated polyplexes and eglin 5 (EG5) siRNA, the

Table 11.4 Polyelectrolyte complexes (polyplexes) developed for delivery of nucleic acids to treat various cancers

Carrier system	Targeting ligand	Drug	Cancer cell line	In vitro and in vivo studies	References
Cationizable (oligoethan amino) amide	FA	siRNA	Human cervix carcinoma (KB)	In vitro, in vivo	[182]
	cMBP2, GE11	pDNA	Hepatocellular carcinoma (Huh7), breast cancer cell line (MCF-7)	In vitro, in vivo	[193, 194, 196–198]
	cMBP2	pDNA	Hepatocellular carcinoma (Huh7)	In vitro, in vivo	[192]
	FA	pDNA	Human cervix carcinoma (KB)	In vitro	[222]
	FA	siRNA	Human cervix carcinoma (KB)	In vitro, in vivo	[190]
	FA	siRNA	Lymphocytic leukemia cell line (L1210)	In vitro, in vivo	[35]
	MTX	dsRNA	Human cervix carcinoma (KB)	In vitro	[186]
	MTX	siRNA	Human cervix carcinoma (KB)	In vitro, in vivo	[187]
	I6P7	pDNA	Human primary glioblastoma cell line (U87)	In vitro, in vivo	[199]
	GE11	miRNA-200c, siRNA	Bladder cancer (T24), breast cancer (MDA-MB 231), liver cancer (Huh7)	In vitro	[34]
	Tf, INF7	siRNA	Myelogenous leukemia (K562), human prostate cancer (D145), neuroblastoma (N2a)	In vitro, in vivo	[191]
	Angiopep-2	siRNA	Human glioma cell line (U87)	In vitro, in vivo	[188]
PEG-pAsp(TEP)-Chol	–	mRNA	Pancreatic cancer cell line (BxPC3)	In vitro, in vivo	[212]
PEG-pAsp(DET)-Chol	cRGD	pDNA	Human cervical carcinoma cells (HeLa)	In vitro, in vivo	[209]

(continued)

Table 11.4 (continued)

Carrier system	Targeting ligand	Drug	Cancer cell line	In vitro and in vivo studies	References
PEG-pAsp(DET)	–	pDNA	Colorectal carcinoma (CT26)	In vivo	[213]
VIPER (OEGMA-DMAEMA&DIPAMA-PDSEMA)	–	pDNA	Human cervix carcinoma (KB), lung adenocarcinoma (A549R	In vitro, in vivo	[214]

Abbreviations:
cMBP2 cMET-binding peptide; *cRGD* cyclo(Arg-Gly-Asp-D-Phe-Cys); *DIPAMA-PDSEMA* poly (2-diisopropylaminoethyl methacrylate)-co-poly(pyridyl disulfide ethyl methacrylate); *FA* folic acid; *GE11* EGFR peptide ligand; *I6P7* interleukin-6 receptor binding peptide; *INF7* influenza virus hemagglutinin-derived fusogenic peptide ligand to enable endosomal escape; *MTX* methotrexate; *OEGMA-DMAEMA* poly(oligo(ethylene glycol) monomethyl ether methacrylate)-co-poly (2-(dimethylamino)ethyl methacrylate); *PEG-PAsp(DET)* poly(ethylene glycol)-polyaspartamide-aminoethylene-diethylenetriamine; *PEG-PAsp(DET)-Chol* poly(ethylene glycol)-polyaspartamide-aminoethylene-diethylenetriamine-cholesterol; *PEG-PAsp(TEP)-Chol* poly(ethylene glycol)-polyaspartamide-aminoethylene-tetraethylenetriamine-cholesterol; *Tf* transferrin; *VIPER* virus-inspired polymer for endosomal release

enhanced antitumoral potency resulted in 50% of recurrence-free survival of mice [187].

The group of Jiang tested an OAA-based oligomer for siRNA delivery into glioma cells with tumor-cell-triggered siRNA release and high biocompatibility. As a targeting modification for enhancing the cellular uptake, angiopep-2 was chosen since it has high affinity to low-density-lipoprotein-receptor-related protein (LRP), which is overexpressed both on the surface of brain capillary endothelial cells (BCECs) and glioma cells. The angiopep-polyethylene glycol (PEG)/siRNA polyplexes indicated effective glioma-targeting siRNA delivery, and intracellular siRNA release, arising in a notable gene downregulation of over 70% of BAG3 expression, both in U87 cells and upon intravenous delivery in glioma model nude mice without significant toxicity [188].

Lee et al. also used folate receptor targeting in their polyplex system, optimized by co-formulating a folate-PEG-OAA with different lipo-OAAs (optionally tyrosine-modified, for optimizing stability and size) to generate targeted lipopolyplexes (TLPs) with the function of self-stabilizing by cysteine disulfide cross-links [189]. Intracellular distribution and siRNA release kinetics were analyzed. The best performer, TLP1, which was tyrosine tripeptide stabilized, unpacked siRNA after cellular uptake in a sustained manner. An up to fivefold higher intracellular siRNA stability after 4 h was noted compared to other TLPs. Furthermore, IV administration of TLP1 in a subcutaneous FR-positive leukemia (L1210) mouse

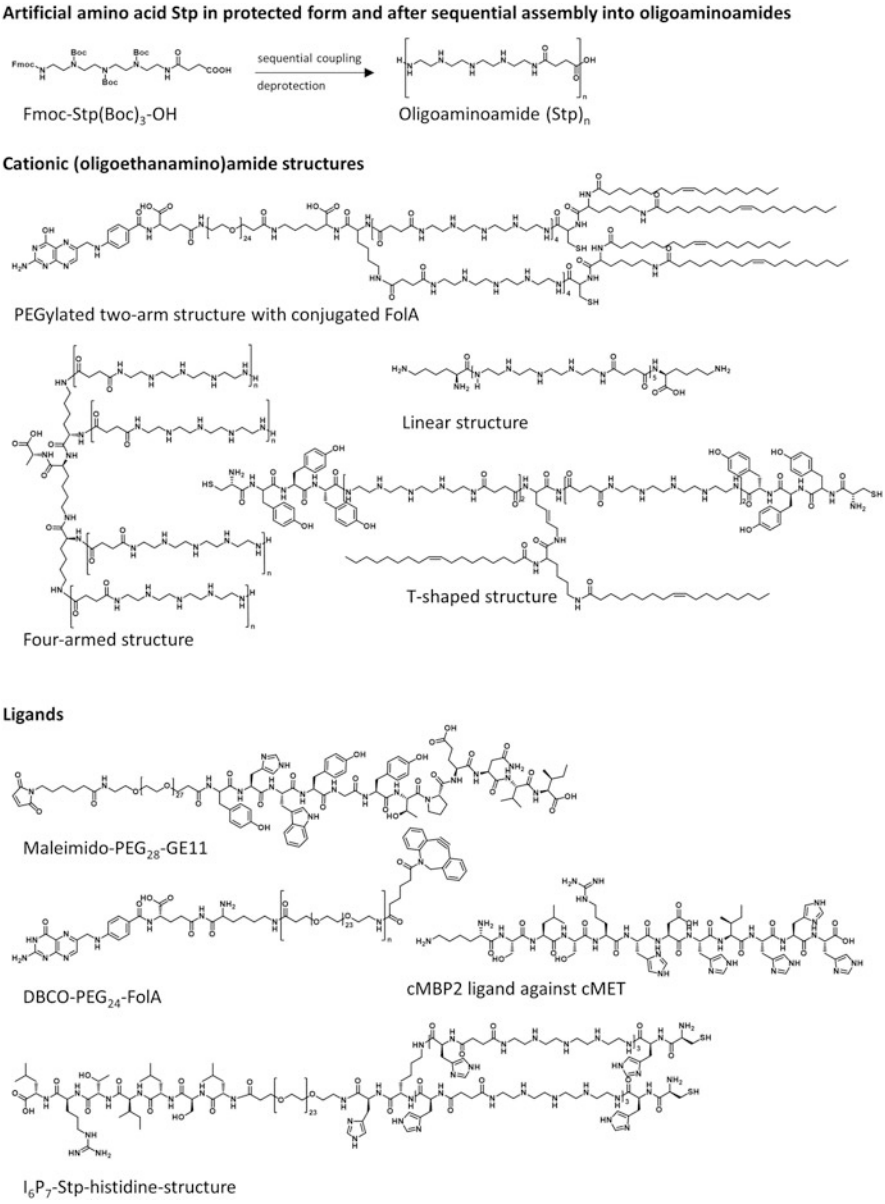

Fig. 11.4 Sequence-defined oligoaminoamides. Chemical structures of the building block Stp, cationic (oligoethanamino)amides, and targeting ligands

model could demonstrate a 65% EG5 gene silencing, measured at the tumoral mRNA level, without detectable adverse effects. These results demonstrated effective tumor-targeted delivery and superior protection of siRNA, through stabilizing the polyplex by introducing disulfide cross-links and tyrosine motifs [190].

Müller et al. advanced a polyplex system based on T-shaped cationizable lipo-OAA containing tyrosine motifs for stabilizing pDNA and siRNA [190, 191] and functionalized the formed polyplex with the targeting peptide ligand GE11 via a PEG linker. The resulting particles demonstrated receptor-mediated uptake into EGFR-positive T24 cells, MDA-MB 231 cells, and Huh7 cells. Furthermore, these formulations led to ligand-dependent gene silencing. RNA interference (RNAi) triggered antitumoral effects were observed for microRNA-200c mimic and EG5 siRNA. The treatment of T24 or MDA-MB 231 cancer cells with the polyplex delivering miR-200c led to decreased proliferation and migration, changes in cell cycle, and enhanced sensitivity toward DOX. In addition, the delivery of EG5 siRNA into Huh7 cells resulted in antitumoral activity, triggered by a loss of mitotic spindle separation and formation of mono-astral spindles [34]. Furthermore, Zhang et al. likewise used the T-shaped sequence-defined polycationic lipo-OAA described in [191] as a multifunctional transferrin receptor (TfR)-targeted siRNA delivery system. The created siRNA lipopolyplex cores were surface modifying with PEG-linked transferrin (Tf) for receptor targeting and the endosomolytic peptide INF7 for an efficient cytosolic release of the siRNA. The effect of Tf-INF7 polyplex internalization and target gene silencing was displayed for TfR overexpressing tumor cell lines (K562, D145, and N2a). As cargo EG5 siRNA was chosen, which led to a block of tumor cell growth and triggered apoptosis. Competition experiments with excess of Tf demonstrated TfR target specificity, showing the effectiveness of this targeting approach. Moreover, in vivo distribution studies illustrated enhanced tumor residence of siRNA in N2a tumor-bearing mice with the Tf-INF7 as compared to the untargeted version of this polyplex, but also reduced stability, which limited the in vivo performance of those polyplexes.

Since cMET is overexpressed on the cell surface of a variety of tumors, Kos et al. used cMET binding peptides (cMBP) as a pDNA polyplex targeting units. Sequence-defined oligomers, with a cationic (oligoethanamino) amide core containing terminal cysteines for redox-sensitive polyplex stabilization, and a monodisperse PEG layer for surface shielding were used as carriers. In addition, histidine units were implemented in the cationic core for promoting the endosomal escape. A cMET-overexpressing DU145 and Huh7 cell model was used. The resulting oligomers using cMBP2 as targeting ligand showed greatly increased cellular uptake and gene transfer over non-targeted control sequences, confirming the efficacy and target specificity of the pDNA polyplexes. The polyplex platform created in this way successfully demonstrated pDNA condensation, serum stability, and receptor-specific luciferase marker gene transfer in vivo in a Huh7 mouse model, both after local intratumoral or intravenous systemic injection. For systemic delivery improved compaction of pDNA by inclusion of a second three-arm oligomer was critical [192].

Urnauer et al. have successfully applied the same system to compact pDNA encoding sodium iodide symporter (NIS), a well-defined theranostic gene, which can target different cancer types, allowing noninvasive imaging of functional NIS expression and therapeutic radioiodide application [193–196] in a sequence-defined polymer made of PEG and a cationizable OAA. As targeting ligand, cMBP2 (cMET-targeting) or GE11 (EGFR-targeting) was used [197, 198]. In the in vivo mouse

model, three cycles of polyplex and radioiodide (^{131}I) application significantly reduced tumor growth and prolonged survival of mice [197]. These data demonstrated the potential of cMET-targeted sequence-defined polymers combined with the unique theranostic function of NIS.

The group of Huang [199] reported the use of interleukin-6 receptor (IL6R) binding I6P7 peptide as a cascade-targeting ligand for non-viral gene delivery. Also here the polymeric foundation consisted of two sequence-defined OAAs, an untargeted three-arm oligomer structure mixed with an PEGylated, I6P7 ligand conjugated oligomer. Both oligomers again contained (Stp)-histidine units and cross-linking cysteines [185, 200]. The resulting polyplexes (I6P7-Stp-His/pDNA) were able to mediate higher gene expression in U87 cells than in healthy human astrocytes due to I6P7, which provides BBB-crossing functions, subsequent glioma targeting, and tumor inhibition effects. The transport across the BBB and specific glioma targeting could be demonstrated in vitro in a transwell bEnd.3 cell model, resulting in transfection of underlying U87 cells and in vivo in glioma-bearing mice. Moreover, targeted delivery of pDNA encoding inhibitor of growth 4 (pING4) significantly prolonged the survival time of orthotopic U87 glioma-bearing mice [199].

Klein et al. [35] established polyplexes based on the complexation of siRNA with the azido-functionalized sequence-defined cationizable lipo-oligomer, which contained two cholanic acids attached to an OAA backbone in T-shape configuration. The formed nanoparticles got additionally surface-functionalized by click chemistry with various folate-conjugated DBCO-PEG agents to provide a targeting and stealth effect. Testing targeting efficiency, cellular uptake, and gene silencing in vitro revealed bivalent DBCO-PEG24-FolA as the lead surface-functionalizing structure. Additionally, in vivo mouse experiments verified an extended biodistribution and intratumoral delivery in the FR-positive L1210 cell model. Furthermore, intravenous injection of analogous therapeutic EG5 siRNA (directed against the kinesin spindle motor protein eglin-5) lipopolyplexes mediated tumoral EG5 mRNA knockdown by ~60%. In combination with nature-derived tubulin inhibitor pretubulysin, the survival rate of aggressive leukemia-bearing mice was prolonged without apparent side effects. Analogous T-shaped lipo-oligomer polyplexes were successfully formulated for EG5 siRNA/methotrexate co-delivery [201], pretubulysin/methotrexate co-delivery [202], EG5 siRNA/pretubulysin co-delivery [203], and the delivery of a dual antitumoral conjugate of siRNA with the pro-apoptotic peptide KLK [204] into tumors. For this purpose, also novel targeting ligand conjugates, including EGFR targeting DBCO-PEG-GE11 and IL4R targeting DBCO-PEG-AP1 [204], were designed.

The group of Kataoka developed a polymeric carrier system based on self-assembly of a PEG-polyamino acid block copolymer for the delivery of pDNA, siRNA, and mRNA [205–208]. The functional polyamino acids possess the capacity of pH-responsive membrane destabilization, allowing endosomal escape of the polyplex into the cytoplasm. Ge et al. advanced this concept by installing the cyclic RGD peptide as ligand, in order to facilitate targeted pDNA delivery to the tumor site as well as promoting cellular uptake and intracellular trafficking behaviors. The

created cRGD-PEG-pAsp(DET)-cholesteryl polyplexes were tested in an subcutaneous fibrotic BxPC3 human pancreatic cancer mouse model, achieving improved accumulation at the tumor site and potent tumor growth suppression by efficient gene expression of antiangiogenic protein (sFlt-1) at the tumor site [209]. Furthermore, Chol conjugation displayed a favorable stabilizing effect [210].

In regard to mRNA delivery, in vitro analyses using specific TLR-expressing HEK293 cells confirmed that the polyplex inclusion prevents mRNA from the recognition by Toll-like receptors (TLRs), showing the capability of this system to prevent triggering the immune response, normally caused by naked mRNA [211]. On this basis Uchida et al. created a mRNA delivery system, based on PEG-polycation block copolymers with conjugated cholesterol (Chol) moieties for an increased polyplex stability by hydrophobic interactions. As a cationic segment, polyaspartamide with four aminoethylene repeats in its side chain (PAsp(TEP)) was selected [205–208], since it helps to increase the nuclease resistance and high protein expression from the mRNA. The IV administration of PEG-pAsp(TEP)-Chol polyplexes for the delivery of mRNA encoding an anti-angiogenic protein (sFlt-1) in the BxPC3 pancreatic cancer subcutaneous inoculation mouse model led to enhanced blood retention of mRNA in comparison to polyplexes without Chol. Moreover, efficient sFlt-1 protein expression in tumor tissue resulted in remarkable inhibition of the tumor growth. In contrast, polyplexes without Chol failed to show a detectable therapeutic effect [212].

Another approach to use the PEG-pAsp(DET) polymeric system as cancer therapeutic has been described by Furugaki et al. [213]. They investigated the delivery of genes encoding the tumor-associated antigen squamous cell carcinoma antigen recognized by T cells-3 (SART3), adjuvant CD40L, and granulocyte macrophage colony-stimulating factor (GM-CSF) as a DNA vaccine platform in mouse tumor models with different types of major histocompatibility antigen complex (MHC).The triple gene vaccine (SART3/CD40L + GM-CSF gene-loaded polyplex) significantly extended the survival of mice with peritoneal dissemination of CT26 cells. Moreover, long-term surviving mice showed complete rejection when re-challenged with CT26 tumors, showing a successful inhibition of growth and metastasis of tumor cells [213].

The group of Pun reported a synthetic polymer designed to mimic viral mechanism of delivery named VIPER (virus-inspired polymer for endosomal release) [214]. The polymer is composed of a hydrophilic cationic block and a pH-sensitive block. The hydrophilic block is synthesized out of poly(oligo(ethylene glycol) monomethyl ether methacrylate)-co-poly(2-(dimethylamino)ethyl methacrylate) (p(OEGMA-DMAEMA)). Moreover, the pH-sensitive block is formed out of poly(2-diisopropylaminoethyl methacrylate)-co-poly(pyridyl disulfide ethyl methacrylate) (p(DIPAMA-PDSEMA)). The VIPER polyplex system was tested in vitro and in vivo on KB and A549 tumor models. The results demonstrated that VIPER can efficiently mediate both in vitro and in vivo gene transfer, showing superior efficiencies compared to the commonly used BPEI agent [214].

In sum, polyelectrolyte complexes (PECs) of therapeutic polyanionic nucleic acids with cationic polymers polyplexes are a most suitable delivery form, because

the carrier also can condense the nucleic acid cargo into smaller NPs and protect it against biodegradation. Polyplexes have been evaluated in ex vivo and in vivo clinical cancer studies [215–217], but up to now not yet approved for therapy.

11.6 Polymer Conjugates

In contrast to polyanionic nucleic acids, most drug and protein cargos cannot directly build polyplexes. Direct covalent conjugation to polymers appears as more suitable delivery form. Polymer conjugates have been intensively reviewed in other reports [10–12]; therefore this section only briefly reviews the subject of polymer-protein delivery.

PEG-protein conjugates present one of the first and most important class of polymer therapeutics [218]. In comparison to small molecule drugs, site-specific conjugation with therapeutic proteins requires refined technologies. Recently, the group of Hua Lu [219] developed a very elegant protein-poly(amino acid) (P-AA) conjugation method by installation of two "chemical handles," including a thioester for native chemical ligation and a polyglycine nucleophile for sortase A-mediated ligation, at both ends of the P-AAs. P-AAs were based on $p(EG_3Glu)_{20}$, i.e., triethylene glycol ester of polyglutamate. In the following, they demonstrated that macrocyclization of interferon-P-AA conjugates improved the tumor retention, penetration, and antitumor efficacy of interferon [220].

Furthermore, the researcher demonstrated that this kind of site-specific polypeptide conjugation which they termed "PEPylation" reduces the risk of anti-conjugate immune responses and resultant antibody drug clearance (ABC) as was observed for the standard PEGylation proteins (interferon or human growth hormone) [221].

Zhang et al. [222] evaluated a library of 16 preselected sequence-defined oligoaminoamides for the delivery of the intracellularly active protein ribonuclease A, which elicits tumor cell killing via intracellular RNA digestion. The protein cargos were attached via bioreducible disulfide bonds. All oligomers contained PEG as a shielding unit and FA as targeting ligand. Folate-receptor-positive KB carcinoma cells were chosen as cancer model, validating both effective endolysosomal escape and subsequent intracellular transport, which was additionally verified using nls-EGFP. The investigation of structure-activity relationships elucidated that the incorporation of oleic acids play a vital role in the enhanced intracellular protein delivery, by facilitating membrane active characteristics for cytosolic delivery and efficient protein transduction, triggering potent tumor cell killing [222, 223]. Liu et al. [224] developed an analogous system with a different, traceless conjugation technique; an AzMMMan click linker [225] was applied for bioreversible protein linkage, which is cleaved under intracellular endosomal acidic conditions, releasing the intact unmodified protein.

11.7 Bioimaging and Theranostic Nanosystems

Improved pharmacokinetics and tumor accumulation will be a prime task in the optimization of the mentioned polymeric drug delivery system. Far less standardization will be possible with regard to the individual patient. Here, the heterogeneity of tumors, tumor stroma, and size dependence of vascularization are an open question with high variability within the same patient and between different patients [2, 226–228]. This uncertainty poses a formidable delivery challenge, which requires novel approaches such as performing tumor imaging before administration of the therapeutic nanoagent. In the ideal situation, functional bioimaging would detect and confirm the molecular target (such as a tumor-specific target receptor) and the accessibility of the tumor (across vasculature, stroma) with a diagnostic nanoagent, which would be harmless and would generate no side effects in non-target organs. The functional results from such a bioimaging would indicate the suitability of nanoagents. In case of alternative options, the most suitable nanoagent (size, surface modification) might be selected. For example, Cabral and colleagues [78] compared in preclinical mouse models the accumulation and antitumoral activity of long-circulating, drug-loaded polymeric micelles with diameters of 30, 50, 70, and 100 nm. All polymer micelles penetrated highly permeable tumors, but only the 30 nm micelles could penetrate poorly permeable pancreatic tumors and showed antitumor effects in a mouse model of this type of cancer. The researchers also showed that the delivery of the larger micelles could be enhanced by using a TGF-beta inhibitor for increased tumor permeability. Analogous treatments for reducing tumor stroma and improved vascular access had been developed by Jain and coworkers [229–231].

Theranostic nanoagents present the special case where the same nanosystem can be used as nontoxic diagnostic and subsequently as antitumoral therapeutic tool. Spitzweg and collaborators applied tumor-targeted pDNA polyplexes encoding sodium iodide symporter (NIS) as a well-defined theranostic gene [193, 194]. Functional NIS expression can be detected using the diagnostic radioisotope iodide ^{123}I by scintigraphy, or using ^{124}I-/^{18}Ftetrafluoroborate for positron emission tomography. Using three cycles of polyplex and therapeutic radioiodide^{131}I application significantly reduced tumor growth and prolonged survival of mice in several models [195–197]. Urnauer et al. also performed a dual targeting approach with LPEI-PEG2kDa-based polymer backbones and cMBP and GE11 as targeting ligands for c-MET and EGFR, respectively. Delivering NIS to hepatocellular cancer (orthotopic Huh7 cells), an increase of tumor-specific transduction efficiency of dual-targeted polyplexes compared to single-targeted polyplexes was demonstrated, resulting in a significant reduction of tumor growth [232].

Numerous polymeric nanoagents have been designed for multifunctional imaging and theranostics of cancer; the current section provides only a snapshot of activities [233, 234]. For pre-selection of cancer patients, integration of imaging properties into nanotheranostics should facilitate clinical translation and personalized nanomedicine administration. Imaging modalities include magnetic resonance

imaging, incorporating superparamagnetic iron oxide nanoparticles (SPION) as MRI contrast agent into drug formulations [235, 236]. Ultrasound irradiation has been applied, with low-power diagnostic or high-power therapeutic irradiation, for example, using theranostic polymer microcapsules composed of hydrogen-bonded multilayers of tannic acid and poly(N-vinylpyrrolidone) that produce high imaging contrast and deliver the anticancer drug doxorubicin upon irradiation [237]. Optical imaging with near-infrared (NIR) light enables deep tissue penetration and the design of NIR-activatable polymeric nanoformulations for combined imaging and therapy of cancer [238]. NIR agents include standard small-molecule dyes but also polymer-coated quantum dots [239] or carbon nanodots for cancer-targeted photothermo-chemotherapy [240, 241].

Tian and colleagues designed tumor-targeted polypyrrole-bovine serum albumin-indocyanine green nanoparticles (SP94-PPy-BSA-ICG) for the detection by photoacoustic and near-infrared (NIR) fluorescence imaging and photothermal ablation of hepatocellular carcinoma (HCC) [242]. The NPs contained ICG for NIR and photoacoustic imaging, and PPy as photothermal and photoacoustic contrast agent. Based on the HCC targeting peptide SP94, a higher tumor accumulation was observed, and the NPs could effectively kill the tumor through photothermal therapy upon a single laser irradiation event. In further work, the same research group generated tantalum oxide (TaOx)-based core/shell NPs for triple-modality image-guided chemo-thermal therapy of esophageal carcinoma [243]. Hollow TaOx NPs encapsulating PPy and doxorubicin in the core and NIR dye800 conjugated on the shell enabled multimodal imaging including computed tomography (for the preliminary location of the tumor), photoacoustic (for the anatomical localization of the tumor), and fluorescence imaging (for real-time monitoring of the tumor margin) as well as pH- and thermal-sensitive drug release. Comparable chemo-thermal therapeutic efficacy of intravenous and aerosol administration was noted, with the latter probably more suitable for clinical therapy of esophageal carcinoma.

11.8 Conclusion

In the field of polymeric NP systems for cancer therapy, extensive studies have been conducted for effective and tumor-specific drug delivery. Studies demonstrate that polymer nanoparticles may become an important antitumoral treatment modality, also confirmed by the fact that some polymer-based systems are already in clinical use for delivering anticancer agents. This was made possible by important advances in this field including controlled stability, high carrier capacity, the ability to load the NP with both hydrophilic and hydrophobic anticancer agents, controlled drug release, and the possibility to deliver multiple drugs at the same time. One of the current main goals is to further improve their pharmacokinetics and rates in tumor accumulation. Another aim is the development of novel bioimaging concepts, for classifying patients in regard to the heterogeneity and vascular penetrability of tumors. Theranostic strategies, combining a diagnostic nanosystem for bioimaging

as selection basis for a subsequent therapeutic nanosystem, as well as modulation of tumor stroma and vasculature, are encouraging directions for medical use.

Surface modification enables control of the transport kinetics and biodistribution of cargo and therefore enhances specific drug delivery at the tumor level while reducing toxicity to normal tissues. On the one hand, several ligands (e.g., TfR ligands, CPPs, LDLR ligands, integrins, carbohydrates (lectin ligands), EGFR ligands, folate) against various tumor-specific or associated markers with high binding affinity are extensively studied as efficient drug delivery tools in tumor targeting. On the other hand, the versatile polymer chemistry allows to modulate the properties of the polymeric matrix in a dynamic fashion. Thus, high therapeutic load and environment-triggered release [3, 244, 245] can be realized.

Nevertheless, the safety of polymeric nanocarriers is an additional important consideration, which needs to be assessed before proceeding to clinical study. In addition, complicated production designs may represent a hindrance for the translation into clinical use. In this regard, future considerations must demonstrate reliability and appropriateness to achieve the advantageous invention and evolution of these polymeric nano-platforms, by substantially improving reproducibility of production, enabling upscaling, clear mechanisms involved in the preparation and loading of anticancer agents, generation and control of membrane permeability, targeted delivery, and release of products on demand. This development of the optimization will be another step forward to explore the full potential of polymeric NP systems designed for tumor targeting.

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