Drug-loaded PEG-PLGA nanoparticles for cancer treatment

Dan Zhang1,2†, Lin Liu1†, Jian Wang1, Hong Zhang1, Zhuo Zhang1, Gang Xing3, Xuan Wang3* and Minghua Liu1*

1Department of Pharmacology, School of Pharmacy, Southwest Medical University, Luzhou, China, 2Pharmaceutical Department of Traditional Chinese Medicine, School of Pharmacy, Southwest Medical University, Luzhou, China, 3Department of Gastroenterology, The Affiliated Hospital of Southwest Medical University, Luzhou, China

Nanoparticles based on single-component synthetic polymers, such as poly(lactic acid-co-glycolic acid) (PLGA), have been extensively studied for antitumor drug delivery and adjuvant therapy due to their ability to encapsulate and release drugs, as well as passively target tumors. Amphiphilic block co-polymers, such as polyethylene glycol (PEG)-PLGA, have also been used to prepare multifunctional nanodrug delivery systems with prolonged circulation time and greater bioavailability that can encapsulate a wider variety of drugs, including small molecules, gene-targeting drugs, traditional Chinese medicine (TCM) and multi-target enzyme inhibitors, enhancing their antitumor effect and safety. In addition, the surface of PEG-PLGA nanoparticles has been modified with various ligands to achieve active targeting and selective accumulation of antitumor drugs in tumor cells. Modification with two ligands has also been applied with good antitumor effects, while the use of imaging agents and pH-responsive or magnetic materials has paved the way for the application of such nanoparticles in clinical diagnosis. In this work, we provide an overview of the synthesis and application of PEG-PLGA nanoparticles in cancer treatment and we discuss the recent advances in ligand modification for active tumor targeting.

KEYWORDS
PEG, PLGA, nanoparticles, antitumor therapy, targeted modification, drug delivery

1 Introduction

Tumor malignancies are the second leading cause of death worldwide and their treatment remains expensive and complex (Wiśniewski et al., 2020). Tumor drug therapies mainly include treatment with cytotoxic small molecules, drugs targeting genes or other molecules, as well as active substances of traditional Chinese medicine (TCM). Although more than half of the 170 drugs used for cancer treatment target specific molecules (Levêque and Becker, 2019), chemotherapy and adjuvant TCM treatment are also widely used in clinical practice due to their relatively low price and good therapeutic effect. However, current therapeutic agents suffer from low bioavailability, rapid elimination in vivo, high toxicity to normal host cells, and low retention at the tumor site. Peptides used in...
extensive vascular surfaces in the tumor tissue, where there is no effect, NPs can be retained within the rich blood vessels and on the tumors: due to the enhanced permeability and retention (EPR) surface properties make them suitable for passive targeting of solid agent composite nanomaterials to some extent (Xu et al., 2015). Also improves the biocompatibility of PLGA-based contrast magnetic nanoparticles, PEGylation reduces neurotoxicity and (Mitchell et al., 2021). In addition, their particle size and unique surface properties make them suitable for passive targeting of solid tumors: due to the enhanced permeability and retention (EPR) effect, NPs can be retained within the rich blood vessels and on the extensive vascular surfaces in the tumor tissue, where there is no lymphatic reflux to clear them away. For instance, NPs based on the US Food and Drug Administration (FDA)-approved poly (lactic acid-co-glycolic acid) (PLGA) have been widely used to encapsulate almost all types of antitumor drugs, offering good biodegradability, minimal systemic toxicity, and high bioavailability (Khan et al., 2016a). However, their application is limited because intravenously administered PLGA NPs are easily opsonized and rapidly cleared by the reticular endothelial system (Noori Koopaei et al., 2014).

In order to improve the properties of PLGA NPs and achieve long-term therapeutic effects, polyethylene glycol (PEG) has been conjugated with PLGA to construct a new type of amphiphilic block co-polymer nanoparticle, PEG-PLGA NPs. Compared with unPEGylated PLGA nanoparticles, PEGylated PLGA nanoparticles showed a characteristic improvement in the symptoms of multiple sclerosis in mice (Li P. Y. et al., 2021), indicating their potential to improve immune tolerance. Pegylated lipid-PLGA hybrid NPs can significantly reduce the fusion phenomenon of nanoparticles during storage, and further improve the internalization of cell uptake experiments while improving stability (Hu et al., 2015). In studies of PLGA-based magnetic nanoparticles, PEGylation reduces neurotoxicity and improves the stability of the loaded therapeutic DNA in primary hippocampal neurons (Cai et al., 2019). PEGylation also improves the biocompatibility of PLGA-based contrast agent composite nanomaterials to some extent (Xu et al., 2015). The novel preparation showed improved drug encapsulation efficiency and controlled release, especially of chemotherapeutic drugs, active TCM substances, and gene-targeting drugs. In addition, PEG-PLGA NPs exhibited high stability, good bioavailability, and enhanced passive targeting ability by the EPR effect, which promoted the targeted accumulation of the drug at the tumor site and improved its safety.

The surface of PEG-PLGA NPs has also been modified with various ligands, such as glycyrhetinic acid, chondroitin sulfate, alendronate, polyethylenimine, iRGD (the arginine-glycine-aspartate-aspartate peptide), and estradiol, in order to allow the NPs to target tumors not only passively but also actively. Extensive studies on the mechanism of highly invasive and metastatic tumors have revealed a large number of abnormally expressed proteins, such as cell adhesion molecules, that can serve as new targets for PEG-PLGA NPs. Cell adhesion molecules are a general class of cell surface transmembrane proteins that mediate cell-cell and cell-extracellular matrix adhesion, especially in tumors. The epithelial cell adhesion molecule (EpCAM) is highly expressed in tumors and it helps regulate the epithelial-mesenchymal transition, giving it a key role in the invasion and metastasis of tumor cells; this molecule can bind specifically to EpCAM aptamer on NPs (Fagotto and Aslemarz, 2020). CD44 is also upregulated in several tumor cell types, and it serves as a marker of cancer stem cells; it can bind specifically to hyaluronic acid on NPs (Chen et al., 2018). The arginine-glycine-aspartate-aspartate peptide on NPs binds with high affinity to the integrin receptor, which is abundantly expressed on certain tumor types; the peptide can then be internalized, taking the NP and its drug cargo inside the target cells (Davoodi and Shafiee, 2022). The folate receptor is overexpressed in certain tumor types such as ovarian cancer and non-small cell lung cancer, and this has been exploited in several approaches to develop high-affinity folates for targeted cancer treatment (Ledermann et al., 2015), as well as folate conjugates for chemotherapy, photothermal therapy, and diagnostic imaging (Li et al., 2015; Liu et al., 2018). Another study showed that biotin, a safe water-soluble vitamin, can bind strongly to biotin receptors and the surface of pharmaceutical preparations, showing great potential as an active targeting strategy for cancer treatment (Wang et al., 2020). Furthermore, the great demand of tumor cells for iron leads them to overexpress transferrin receptors on their surface, which might provide another strategy for targeted therapy (Luck and Mason, 2013).

In this review, we discuss the formulation principles and properties of PEG-PLGA NPs and summarize the recent advances in their modification and application as drug delivery systems for targeted cancer treatment (Figure 1). The synthesis provided here may guide the development of new antitumor formulations with improved in vivo pharmacokinetics, enhanced passive and active targeting, as well as high drug efficiency for effective precision medicine.

## 2 PEG-PLGA NPs

### 2.1 Origin of drug-loaded PEG-PLGA NPs

Due to its great biocompatibility and biodegradability, PLGA has been widely used in the preparation of NPs (Lakkireddy and Bazile, 2016). Although PLGA NPs are good carriers for
hydrophilic and hydrophobic drugs, their application is limited due to protein opsonization and rapid clearance by the reticuloendothelial system. Adagen was the first PEGylated protein drug approved by the FDA as a treatment for severe combined immunodeficiency (Suk et al., 2016). When PEG covalently binds to the drug surface, it will block antigen determinants to affect antigen-antibody binding to inhibit the immunoreaction. The immunogenicity of ricin against anti-ricin serum can be reduced through PEG modification, which covers epitopes and receptors involved in immune recognition (Hu et al., 2002). PEGylating a genetically engineered form of alginate lyase significantly reduced its ability to be recognized by antibodies from New Zealand rabbits and humans (Lamppa et al., 2011). Similarly, PEGylating porcine follicle-stimulating hormone protected the hormone from immune recognition (Uchiyama et al., 2010). The PEG surface barrier can also protect the drug from enzymatic degradation and rapid elimination by the kidney, prolonging the half-life of the drug in vivo. PEGylating recombinant human interleukin-11 (IL-11) not only enhanced its pharmacological activity, but also prolonged its retention time in plasma by reducing the liver and kidney clearance of IL-11 (Takagi et al., 2007) (Menkhorst et al., 2009). Modifying PLGA NPs with PEG improves their surface hydrophilicity and prolongs circulation time (Noori

FIGURE 1
Schematic of applications of PEG-PLGA nanoparticles. EPR, enhanced permeability and retention enhanced permeability and retention; PEG, polyethylene glycol; PLGA, poly(lactic acid-co-glycolic acid); siRNA, short interfering RNA.
Koopaei et al., 2014), giving them substantial promise as drug carriers (Haggag et al., 2018; Dunn et al., 2019). These NPs consist of a PEG shell and a PLGA core that can effectively encapsulate hydrophilic and hydrophobic drugs (Figure 2).

2.2 Preparation of PEG-PLGA NPs

Nanoprecipitation and double emulsion-solvent evaporation are the two main methods for the synthesis of PEG-PLGA NPs; they take advantage of the self-assembly of PEG and PLGA at a specific ratio and temperature. Below we describe only the basic synthetic routes and characteristics of the two methods, since we cannot cover the large variability in excipients, component proportions and reaction conditions that have been explored.

2.2.1 Nanoprecipitation

Nanoprecipitation is a simple preparation method for NPs with narrow particle size distribution that requires low amounts of surfactant, generates few toxic products, and can be performed on a large scale. For the preparation of PEG-PLGA NPs, PEG and PLGA are dissolved in a suitable solvent, mainly acetone, and then added to an aqueous phase to complete their self-assembly. The solvent is finally removed by dialysis or volatilization, and PEG-PLGA NPs are collected. Using this method, PEG-PLGA NPs loaded with a manganese (II) complex were prepared and they showed excellent encapsulation and drug-loading efficiency, leading to good therapeutic effect against breast cancer stem cells (Eskandari and Suntharalingam, 2019). In another study, honokiol-loaded NPs prepared by nanoprecipitation were modified to obtain nanocarriers with high-loading capacity, which enhanced anti-breast cancer activity in vitro and in vivo (Haggag et al., 2020). Nevertheless, further research is still needed to clarify how the choice of organic or aqueous phase, encapsulated drug, temperature, pH, and sequence of reagent addition influence drug loading and encapsulation into NPs (Almoustafa et al., 2017).

2.2.2 Double emulsion-solvent evaporation method

In the double emulsion-solvent evaporation method, PEG, PLGA and drug are added into an organic solvent (oil phase) to prepare a water-in-oil (W/O) emulsion. The resulting emulsion is then added into a water phase, and the mixture is homogenized by sonication to obtain a W/O/W emulsion (Chen et al., 2019; Shen and TanTai, 2020). Evaporation of the organic solvent followed by filtration yields drug-loaded PEG-PLGA NPs.

Similar to the O/W single emulsion-solvent evaporation method, this approach is used to encapsulate proteins and hydrophilic drugs and limit their diffusion out of the NPs, thereby improving entrapment efficiency and sustained release (Zhang et al., 2014). For instance, this method was used to prepare salidroside-loaded PEG-PLGA NPs with high entrapment efficiency by adjusting the glycolic acid/lactic acid molar ratio and the molecular weight of PLGA. The resulting preparation showed low polydispersity index, high zeta potential, and good release and cytotoxicity properties in vitro, indicating that the behavior of PEG-PLGA NPs strongly depends on composition and choice of raw materials (Fang et al., 2014). Endostar-loaded PEG-PLGA NPs were also prepared by double emulsion-solvent evaporation, and they showed sustained and controlled drug release properties as well as specific tumor targeting ability in vivo (Hu and Zhang, 2010).

3 Application of drug-loaded PEG-PLGA NPs in cancer treatment

3.1 Chemotherapeutic applications

Although chemotherapy remains the main treatment approach for cancer, chemotherapeutic drugs suffer from low targeting ability, low cytotoxicity, fast elimination, serious side effects, and high drug resistance. PEG-PLGA NPs have emerged as a novel formulation with great biocompatibility and non-immunogenicity that can improve the solubility, stability, and safety of chemotherapeutic drugs for the treatment of various cancer types (Table 1). For example, paclitaxel (PTX)-loaded PEG-PLGA NPs rapidly prepared by microwave synthesis showed similar cytotoxicity to Taxol (Dunn et al., 2019). Satisfactory pharmacokinetic and pharmacodynamic results have also been reported for docetaxel...
| Treatment type | Preparation of drug loaded PEG-PLGA nanoparticle | Payload | Treatment model | Administration mode | Advantages | Reference |
|----------------|-----------------------------------------------|--------|----------------|-------------------|-----------|-----------|
| Chemotherapy   | Microwave synthesis                           | PTX    | HeLa cell      | Culture medium    | Unique release and dose-dependent cytotoxicity | Dunn et al. (2019) |
| Chemotherapy   | 3-factor, 3-level Box-Behnken design           | DTX    | SKOV-3 cell; tumor bearing female balb/c mice | Culture medium, intravenous | Higher cytotoxic efficacy and less weight loss | Noori Koopaei et al. (2014) |
| Chemotherapy   | Nanogel mixed system                           | PEGylated Taxol | 4T1-luciferase cells transplanted female balb/c mice | Intravenous | more efficient inhibition the growth | Wei et al. (2013) |
| Chemotherapy   | Modified double emulsion method                | 5-FU   | Solid Ehrlich carcinoma murine | Intraperitoneal injection | Reduction in tumor volume and weight, improvement on sustained release in vitro and anticancer efficacy in vivo | Haggag et al. (2018) |
| Chemotherapy   | Ring opening melt polymerization method, double emulsion method | 5-FU, Chrysin | HT29 human colon cancer cell | Culture medium | Higher growth inhibitory effects, improvement on the therapeutic and functional delivery efficacy | Khaledi et al. (2020) |
| Chemotherapy   | Modified double-emulsion solvent evaporation  | Sorafenib, PDEF | C-26 cell, HEK-293, C-26 cell transplanted balb/c mice | Culture medium, intravenous | Higher entrapment efficiency, better sustained manner, no obvious toxicity | Chen et al. (2019) |
| Chemotherapy   | Modified emulsification solvent evaporation    | Gefitinib, Quercetin | PC-9 cell, PC-9 cell transplanted mice | Culture medium, intravenous | Higher cellular uptake and cell inhibition rates | Shen and TanTai, (2020) |
| Chemotherapy   | Self-assembly of PLGA-PEG-PLGA copolymer micelles, CNDs, and DOX | DOX | HeLa cell, (PC3, human prostate cancer cell line) cell transplanted Female nude mice (BALB/cScic/nu/nu) | Culture medium, Intratumor injection | long-term sustained antitumor activity | Nagahama et al. (2015) |
| Chemotherapy   | Two-step surface functionalization method      | Bendamustine | A549 cell, MCF-7 cell, T47D, PC-3 | Culture medium | Less hemolytic, improvement on stability and anticancer efficacy | Khan et al. (2016) |
| Chemotherapy   | Double emulsion method                         | Endostar | HT-29 cell transplanted BALB/c nude mice | Intravenous | Sustained release, improvement on anticancer activity | Hu and Zhang, (2010) |
| Chemotherapy   | Ring opening polymerization method             | Metformin | SKOV-3 cell | Culture medium | More cytotoxicity in a time- and dose-dependent manner, improvement on anticancer activity | Fazmarzii et al. (2019) |
| Chemotherapy   | Self-assemble in water, nanoprecipitation method | Manganese (II) complex | HMLER-shEcad cells | Culture medium | Improvement on breast cancer stem cells, reduction in toxicity | Eskandari and Suntharlalingam, (2019) |
| Traditional Chinese medicine | Double emulsion method                          | Chrysin | AGS cell | Culture medium | Up regulation of expression of miR-34a, higher solubility, significant inhibitory effect in cell growth | Mohammandian et al. (2015) |
| Traditional Chinese medicine | Modified emulsion of oil in water               | Chrysin, curcumin | SW480 cell | Culture medium | Higher bioavailability and solubility, down regulation of expression of telomerase (hTERT) gene | Bagheri et al. (2018) |
| Traditional Chinese medicine | Double emulsion/solvent evaporation methods     | DIM, EA | Human pancreatic cancer cell line, Chack Chorioallantoic Membrane (CAM) Cancer Implant Model | Culture medium, intramodle injection | More effective suppression of pancreatic cancer cell viability, pancreatic tumor weight, implanted cancer cell viability, and tumor angiogenesis | Mousa et al. (2020) |
| Traditional Chinese medicine | Organic solvent volatilization method           | Ginsenoside, 25-OCH3-PPD | Human prostate cancer cell lines LN CaP | Culture medium, oral | MDM2 oncogene inhibition, steady and sustained release | Voruganti et al. (2015) |

(Continued on following page)
(DTX)- and PTX-loaded PEG-PLGA NPs (Wei et al., 2013; Noori Koopaei et al., 2014). In another study, 5-fluorouracil (5-FU)-loaded PEG-PLGA NPs improved the encapsulation, controlled release, and efficacy of the drug against solid Ehrlich carcinoma, while reducing the drug’s adverse effects (Haggag et al., 2018). PEG-PLGA NPs encapsulating doxorubicin (DOX) (Nagahama et al., 2015), bendamustine (Khan et al., 2016b), Endostar (Hu and Zhang, 2010), metformin (Faramarzi et al., 2019), and manganese (II) complex (Eskandari and Suntharalingam, 2019) have shown good sustained release and anticancer properties in vitro and in vivo. In addition, PEG-PLGA NPs have been used for the co-delivery of chemotherapeutic drugs, such as 5-FU and chrysin or sorafenib and pigment epithelium-derived factor for colorectal cancer therapy, as well as gefitinib and quercetin for lung cancer treatment (Chen et al., 2019; Khaledi et al., 2020; Shen and TanTai, 2020). The co-loaded NPs demonstrated better sustained release performance, targetability, and tumor growth inhibition than single drug-loaded NPs. These results suggest that PEG-PLGA NPs can be used for the synergistic treatment of tumors, while reducing the frequency of drug administration.

### 3.2 TCM therapy

Active TCM substances have attracted increasing attention as antitumor drugs or adjuvant therapy for chemotherapy due to their multi-target ability. However, their short half-life, rapid metabolism, low bioavailability, and poor targeting ability significantly limit their application. Therefore, the encapsulation of such active substances into shells (nanoparticles, liposomes, gels, vesicles, etc.), such as PEG-PLGA NPs, can improve their properties and anticancer efficacy (Table 1). For example, chrysin-loaded PEG-PLGA NPs upregulated miR-34a and showed higher solubility and inhibitory activity than free chrysin against AGS cell growth (Mohammadian et al., 2015). PEG-PLGA NPs co-loaded with chrysin and 5-FU or curcumin also exhibited significant synergistic anticancer effects in colorectal cancer treatment (Bagheri et al., 2018; Khaledi et al., 2020). Similarly, PEG-PLGA NPs carrying both di-indolylmethane and ellagic acid effectively reduced the viability of pancreatic cancer cells and suppressed tumor growth and angiogenesis (Mousa et al., 2020). In addition, PEG-PLGA NPs loaded with ginsenosides showed better oncogene regulation, anticancer synergism, drug uptake, half-life, and safety than the corresponding free drugs (Voruganti et al., 2015). PEG-PLGA NPs co-loaded with drugs and active TCM monomers such as icariin (Alhakamy, 2021), salidroside (Fang et al., 2014), and honokiol (Haggag et al., 2020) have also shown anticancer synergistic effects and sustained release. For instance, lupeol-loaded PEG-PLGA NPs enhanced the sensitivity of hepatocellular cancer to radiotherapy (Xie et al., 2021), which may provide a new research direction for antitumor drug resistance. These results suggest that the encapsulation of existing and newly discovered active TCM substances into PEG-PLGA NPs can promote their application in cancer treatment.

### Table 1 (Continued) Recent applications of PEG-PLGA nanoparticles as drug carriers.

| Treatment type | Preparation of drug loaded PEG-PLGA nanoparticle | Payload | Treatment model | Administration mode | Advantages | Reference |
|----------------|-----------------------------------------------|---------|-----------------|---------------------|------------|-----------|
| Traditional Chinese medicine | Response surface (three-level design) | Icarin | ASPC-1 cell | Culture medium | Higher cytotoxicity and apoptotic potential, arrest of G2-M phase of asc-1 cells; upregulation of caspase-3 | Alhakamy, (2021) |
| Traditional Chinese medicine | Ring open copolymerization of lactide and glycolide, double emulsification method | Salidroside | 4T1 cell; PANC-1; SKOV-3 cell; PC-3 cell; CT26 cell; one human normal cell line (AD293) | Culture medium | Gradually release; significant improvement in vitro antitumor activity of Sal in PANC-1 and 4T1 cancer cell lines; no toxicity on AD293 cells at a concentration (100 μg/ml); higher antitumor efficacy | Fang et al. (2014) |

Abbreviations: DIM-3, 3′-diindolylmethane; DOX-doxorubicin; DTX-Docetaxel; EA-ellagic acid; 5-FU-5-fluorouracil; PEDF-pigment epithelium-derived factor; PEG-polyethylene glycol; PLGA-poly(lactic acid-co-glycolic acid); PTX-Paclitaxel.
TABLE 2 Recent applications of ligand-modified PEG-PLGA nanoparticles as drug carriers for actively targeted cancer therapy.

| Modifying molecule | Modification methods | Target | Payload | Treatment model | Progressiveness compared with non-target preparation | Reference |
|--------------------|----------------------|--------|---------|-----------------|------------------------------------------------------|-----------|
| Folate             | A three-step chemical synthesis | Folate receptors | Saquinavir | PC-3 (human prostate) cells, MCF-7 (human breast) cancer cell lines | Cell experiment: cytotoxicity; cellular uptake | Singh et al. (2015) |
| Folate             | Carbodiimide chemistry | Folate receptors | Sorafenib | BEL7402 cells | Cell experiment: cellular uptake; suppression on cell proliferation; anticancer efficacy; inhibition on the colony forming ability | Li et al. (2015) |
| Folate             | Covalent linkage | Folate receptors | Paclitaxel; indocyanine green; perfluorohexane | MDA-MB231 cells; tumor-bearing mice | Cell experiment: cellular uptake; anticancer effect; Transplant model experiment: accumulation in tumor tissue; targeting ability; microbubble activation; low toxicity | Liu et al. (2018) |
| EpCAM aptamer      | Covalent linkage | Epithelial cell-adhesion molecules | Doxorubicin | A549 cell; SK-MES-1 cell; nude mice bearing SK-MES-1 non-small cell lung cancer xenografts | Cell experiment: cytotoxicity; Transplant model experiment: weight loss; toxicity; tumor inhibition | Alibolandi et al. (2015a) |
| EpCAM aptamer      | Covalent linkage | Epithelial cell-adhesion molecules | Doxorubicin | EpCAM-positive tumor cells (MCF-7) | Cell experiment: cell uptake; internalization; cytotoxicity | Alibolandi et al. (2015b) |
| Transferrin        | Simple amide coupling | TFR | Thymoquinone | A549 cells (TFR overexpression); chick CAM xenograft models; xenograft model in immunosuppressed Balb/c mice | Cell experiment: nanoparticle internalization; p53 up-regulation for apoptosis; Transplant model experiment: anti-cancer activity via controlling the p53/miR-34a/miR-16 axis | Upadhyay et al. (2019) |
| Transferrin        | Maleimide-thiol coupling reaction | TFR | Doxorubicin; tetrahydrocurcumin | Rat C6 glioma cell line; human breast cancer cell line (MCF-7); nude mice bearing glioma xenografts | Cell experiment: cell uptake; synergistic effect of radiotherapy; Transplant model experiment: drug accumulation in the brain | Zhang et al. (2019) |
| Biotin             | Dlink reaction | Biotin receptors | Doxorubicin | 4T1 cells; female Balb/C mice bearing 4T1 cell xenografts | Transplant model experiment: improvement in vivo antitumor efficacy; potential of mitigating toxic effect | Singh et al. (2017) |
| Biotin             | DDC/NHS chemistry method | Biotin receptors | DI | Human cervical cancer Hela cells | Cell experiment: antiproliferative activity for preferential internalization; decreasing the intracellular reactive oxygen species (ROS) level | Luo et al. (2018) |
| A10 aptamer        | Conjugated the RNA aptamer to the terminus of PEG-PLGA | PMSA | TFO | LNCaP cell (PMSA+); BALB/c nude mice bearing a LNCaP cell xenograft | Cell experiment: silenced the AR gene; cytotoxicity; Transplant model experiment: cellular uptake | Jiao et al. (2016) |
| Hyaluronic acid    | Activated carbonyl covalently linked | CD44 molecular | Cisplatin | CD44-over expressing ovarian cancer cell line (SKOV-3); Ehrlich tumor (solid) bearing mice | Cell experiment: cytotoxicity; cellular uptake; Transplant model experiment: antitumor activity | Alam et al. (2017) |
| Glycyrrhetinic acid | Chemical synthesis by a two-step process | Glycyrrhetinic acid receptors | Artesunate | HepG2 cell; Hep3B cell; SMCC-7721 cell | Cell experiment: cytotoxicity; binding affinity; and accumulation in hepatoma cells | Pan et al. (2020) |
| Chondroitin sulfate | PEG-Bis-Amine Link | Chondroitin sulfate receptors | 5-fluorouracil | MCF-7/MDA-MD 231 breast cancer cells | Cell experiment: cytotoxic effect; hemolytic potential | Yadav et al. (2010) |

(Continued on following page)
3.3 Active targeted cancer therapy

NPs have special structure, chemical properties, and passive targeting, which allow them to encapsulate nonspecific or targeted drugs and TCM. Although NPs can enhance the targeting ability of antitumor drugs due to the ERP effect and passive enhanced permeability, they still lack the ability to target malignant cells (Tuwahatu et al., 2018; Barkat et al., 2021). To promote active targeting, the surface of PEG-PLGA NPs has been modified with various ligands, such as folate, aptamer, transferrin, and hyaluronic acid (Table 2).

For example, the 5'-NH2-modified EpCAM aptamer was covalently bound on the surface of DOX-loaded PEG-PLGA NPs, and the modified NPs showed stronger inhibitory activity on tumor growth in nude mice bearing non-small cell lung cancer xenografts and higher cytotoxicity against A549 cells than unmodified NPs (Alibolandi et al., 2015a). In addition, EpCam aptamer-conjugated PEG-PLGA NPs enhanced the cellular uptake and cytotoxicity of DOX against human breast adenocarcinoma cells (Alibolandi et al., 2015a; Alibolandi et al., 2015b). Conjugation of A10 aptamer to PEG-PLGA NPs loaded with triplex-forming oligonucleotides led to specific targeting of prostate cancer cells and inhibition of tumor growth, and the modified NPs silenced the androgen receptor gene more effectively than unmodified NPs (Jiao et al., 2016).

Transferrin has also been added to drug-loaded NPs due to its non-toxicity, biodegradability, and low expression in most normal tissues. For example, thymoquinone-loaded PEG-PLGA NPs modiﬁed with transferrin signiﬁcantly induced cancer cell apoptosis by regulating the p53/miR-34a/miR-16 axis (Jain et al., 2015). In addition, transferrin is known to promote active targeting, the surface of PEG-PLGA NPs has been covalently bound on the surface of DOX-loaded PEG-PLGA NPs (Jiao et al., 2016). In addition, transferrin covalently bound on the surface of DOX-loaded PEG-PLGA NPs (Jiao et al., 2016).

### TABLE 2 (Continued) Recent applications of ligand-modified PEG-PLGA nanoparticles as drug carriers for actively targeted cancer therapy.

| Modifying molecule | Modification methods | Target | Payload | Treatment model | Progressiveness compared with non-target preparation | Reference |
|--------------------|----------------------|--------|---------|-----------------|-----------------------------------------------------|-----------|
| Alendronate        | Multistep synthesis  | Mineral hydroxyapatite | Bortezomib | Female Nod/SCID beige mice injected with Luc+/-GFP + MMIS cells | Transplant model experiment:retention, accumulation; bone homing of targeted | Swami et al. (2014) |
| LFC131             | Covalent bonding of NHS-activated PEG-PLGA nanoparticles | CXCR4 | Sorafenib; metapristone (RU42633) | HCC cell lines (HepG2, Huh7, and SMMC-7721 cells); female BALB/c nude mice injected subcutaneously with human SMMC-7721 cells | Cell experiment: nontargeted levels of drugs; anti-proliferative efficacy; tumor cell apoptosis; accumulation in tumors. Transplant model experiment: inhibitory efficacy on tumor growth | Zheng et al. (2019) |
| PEI                | Postsynthesis of PEG-PLGA nanoparticles | SP94 | TK-p53-NTR | Female nude mice (nu/nu) injected with HepG2-Flac cell | Transplant model experiment: gene-loaded transfer capacity biosafety | Li et al. (2021) |
| iRGD               | Interaction between Mal groups of Mal-PEG-PLGA and the thiod group of iRGD for 24 h | iRGD receptors | Crocomanine815 | MDA-MB-231 cells; MDA-MB-231 cell bearing nude mice-Transplant model experiment: inhibition on tumor proliferation | Cell experiment: targeting ability | Sukumar et al. (2020) |
| E2                 | Covalent conjugation | ER | Docetaxel | ER positive MCF-7 cells; HeLa cells (ER negative); breast cancer model in female Sprague Dawley (SD) rats | Transplant model experiment: cellular uptake in ER positive MCF-7 cells; cytotoxicity; Transplant model experiment: tumor regression | Jain et al. (2015) |
| Pep-E2 (CGEMGWYRC, CGKRR/Cys-Gly-Lys-Arg-Lys) peptide | Emulsion/solvent evaporation method | Interleukin 13 receptor α2; heparan sulfate | Paclitaxel | Human umbilical vein endothelial cells; rat C6 glioma cell line nude mice injected with C6 cells | Cell experiment: cellular uptake; improvement of in vitro antitumor activity in the respect of proliferation, tumor spheroid growth, tube formation, and migration. Transplant model experiment: targeted and accumulated at glioma site | Lv et al. (2016) |

Abbreviations: DDC-dicyclohexylcarbodiimide; D1-15, 16-Dihydrorhodamine 1; ER-estradiol receptors; EpCAM-epithelial cell adhesion molecular; E2-estradiol; NTR-nitroreductase; PEG-polyethylene glycol; PEI-polyethylenimine; PLGA-poly(lactic acid-co-glycolic acid); PMSA-prostate specific membrane antigen; TFO-triplex forming oligonucleotides; TFR-transferrin receptor; TK-thymidine kinase.
accumulation of the drugs and showed good anti-glioma efficacy in vivo (Zhang et al., 2019).

Since biotin receptors are overexpressed in cancer cells, NPs modified with biotin have been developed. Among them, 15,16-dihydotanshione I-loaded PEG-PLGA NPs modified with biotin prevented HeLa cell proliferation by downregulating reactive oxygen species and triggering G2/M phase cycle arrest (Luo et al., 2018). DOX-loaded PEG-PLGA NPs modified with biotin have shown good potential in vitro and in vivo for active, targeted therapy of breast cancer (Singh et al., 2017).

Hyaluronic acid can specifically bind to CD44 molecular receptors, which are involved in regulating specific cell-cell and cell-matrix interactions. Cisplatin-loaded PEG-PLGA NPs modified with hyaluronic acid were internalized to a greater extent than unmodified NPs by human ovarian cancer (SKOV-3) cells overexpressing CD44, and the modified NPs were more toxic against those cells (Alam et al., 2017).

Folate, which is highly expressed in a wide range of tumor cells, was also conjugated to PEG-PLGA NPs, affording nanomaterials with superior cytotoxicity and improved cellular uptake due to the specific binding of folate to the corresponding receptors (Singh et al., 2015). Similarly, folate-decorated PEG-PLGA NPs loaded with sorafenib showed high cellular uptake, antiproliferation activity and antitumor effects against BEL7402 cancer cells (Li et al., 2015).

PEG-PLGA NPs decorated with glycyrrhetinic acid were able to concentrate the drug arsetusine in liver cancer cells (Pan et al., 2020). 5-FU-loaded PEG-PLGA NPs modified with chondroitin bound to the chondroitin sulfate receptors overexpressed on various tumor cell types, leading to higher cytotoxicity and less hemolytic effects than unmodified NPs (Yadav et al., 2010).

Targeting the tumor microenvironment has also emerged as an effective strategy for cancer treatment. For example, alendronate-loaded PEG-PLGA NPs were modified with hydroxyapatite, an abundant mineral in bone tissues with high affinity for bisphosphonates, and the formulation accumulated in bone tumors in vivo (Swami et al., 2014). PEG-PLGA NPs that were co-loaded with sorafenib and metapristone and were conjugated with LFC131, a peptide inhibitor of CXCR4 (Zheng et al., 2019), showed promise in bypassing CXCR4-mediated resistance of hepatocellular carcinoma tumor cells to the widely used drug sorafenib.

Recent studies have investigated the role of modified PEG-PLGA NPs in photoacoustic imaging and photothermal tumor therapy. For example, folate-conjugated PEG-PLGA NPs were co-loaded with indocyanine green, perfluorohexane, and PTX to prepare NPs that could be simultaneously used for photoacoustic and enhanced ultrasound echo imaging as well as for active targeting of tumors overexpressing folate receptors (Liu et al., 2018). PEG-PLGA NPs that were loaded with pH-sensitive croconaine815 and decorated with iRGD showed strong photoacoustic signal enhancement and effectively inhibited tumor growth, serving as a novel strategy for in vivo multiplexed photoacoustic imaging and pH-responsive photothermal therapy (Li S. et al., 2021). In addition, PTX-loaded PEG-PLGA NPs that were decorated with the peptides Pep-1 (CGEMGWVRC) and CGKRK (Cys-Gly-Lys-Arg-Lys) enhanced the antiglioma efficacy of PTX by inhibiting angiogenesis and killing cancer cells (Lv et al., 2016).

3.4 Gene-targeting cancer therapy

Cancer gene therapy uses nucleic acids, oligopeptides and proteins to treat tumors by regulating the expression of related genes. However, these drugs cannot be extensively used in clinical practice due to their fast degradation and easy elimination in the blood (Kim et al., 2016). To address these problems, polymer NPs such as PEG-PLGA NPs have been extensively studied as drug carriers due to their unique size, simple modification, good biocompatibility, stability, and low toxicity (Xin et al., 2017). For example, verapamil-modified PEG-PLGA NPs loaded with SN38 enhanced the expression of apoptosis-related genes (BAX/BCL2) and delayed drug resistance in colorectal cancer cells (Nagheh et al., 2017). PEG-PLGA NPs containing a novel peptide inhibiting the protein “Ras protein-regulator of chromosome condensation 1” may be able to inhibit breast cancer metastasis (Haggag et al., 2019). PEG-PLGA NPs co-loaded with DTX and short interfering RNA targeting the oncogene TUBB3 or co-loaded with sorafenib and metapristone targeting the SDF-1/CXCR4 axis have also shown promise against oncogenes in hepatocellular carcinoma (Zheng et al., 2019; Conte et al., 2021). PEG-PLGA NPs that were loaded with a “thymidine kinase–p53–nitroreductase” triple therapeutic gene and decorated with polyethyleneimine were able to inhibit growth of hepatocellular carcinoma tumors (Sukumar et al., 2020).

4 Biocompatibility, toxicity, and safety of drug-loaded PEG-PLGA NPs

All formulations developed for biomedical or clinical use must be non-toxic and comply with the relevant biosafety regulations, while showing good biocompatibility, especially for intravenous or intraperitoneal injection. Although PLGA and PEG have been approved by the FDA as safe and biodegradable, few studies have explored the safety of PEG-PLGA NPs in vivo for further clinical application. For example, blank PEG-PLGA NPs showed negligible inhibitory effects on the growth of human colon adenocarcinoma SW-480 cells, confirming their good biocompatibility (Dimchevska et al., 2017). PEG-PLGA NPs loaded with bendamustine led to nearly 4-fold lower hemolysis than NPs without the PEG-PLGA matrix,
also indicating good biocompatibility (Khan et al., 2016b). In addition, no significant cytotoxicity was observed for blank PEG-PLGA NPs toward HeLa cells (Luo et al., 2018), while the intraperitoneal injection of blank or honokiol-loaded PEG-PLGA NPs did not cause substantial damage to the liver or kidney of mice with breast cancer tumors (Haggag et al., 2020), further indicating the safety of these nanocarriers in vivo. Similarly, PEG-PLGA NPs loaded with the anticancer ginsenoside 25-OCH3-PPD did not cause histopathology in the liver, kidneys, lungs, spleen, heart, or brain of mice bearing PC3 xenograft tumors (Voruganti et al., 2015), while estradiol-decorated DTX-loaded PEG-PLGA NPs showed negligible cytotoxicity in MCF-7 cells and minimal hepatotoxicity in mice (Jain et al., 2015). A recent study analyzed the plasma activation levels of complement C3 induced by the intravenous injection of PEG-PLGA NPs conjugated with anti-cathepsin K antibodies. The results revealed no significant upregulation of complement C3 after treatment with the modified NPs, suggesting that they do not induce an immune response (Camardo et al., 2020). DOX-loaded PEG-PLGA NPs decorated with biotin showed low hemolytic activity and cytotoxicity and proved to be safe relative to free DOX (Singh et al., 2017). Odoranalectin-modified PEG-PLGA NPs were found to be non-toxic to human lung adenocarcinoma Calu-3 cells and showed negligible toxicity and immunogenicity in the nasal cavity in toads and rats (Wen et al., 2011). PEG-PLGA NPs modified with both Pep-1 and CGKRK peptides were also shown to be non-toxic to major organs in mice (Lv et al., 2016).

Nevertheless, the influence of different formulation ratios, molecular weights, and synthesis methods on the safety of PEG-PLGA NPs has not been adequately explored. It is important to analyze the relationship between the toxicity of NP carriers and their physical characteristics, including polydispersity index, zeta potential, entrapment efficiency, and morphology. In fact, the entire process from raw materials to synthesis and modification of drug-loaded PEG-PLGA NPs should be rigorously optimized to maximize safety before clinical trials. Ultimately, uniform guidelines for synthesizing and formulating PEG-PLGA NPs are needed in order to ensure their efficacy as drug carriers for targeted cancer treatment.

5 Conclusion and prospects

The rapid growth of nanotechnology has led to the emergence of many novel therapeutic methods such as nanodrug delivery systems. The present review shows that amphiphilic block copolymer PEG-PLGA NPs can be safely used as drug nanocarriers that show sustained release properties as well as improved drug bioavailability and stability in vivo. PEG-PLGA NPs modified with ligands can target specific receptors on the tumor surface, enhancing tumor targeting. However, the biocompatibility, toxicity, and safety of these nanocarriers require further research to guarantee their clinical application. New cancer-specific target molecules are constantly being discovered, and studies should continue to explore how to modify NPs in order to recognize tumors. This approach may create new possibilities for precision anti-cancer treatment and diagnostic imaging.

Author contributions

ML, LL, DZ, and XW contributed to conception and design of the study. DZ, LL, JW, HZ, ZZ, and GX organized the database. ML, LL, DZ, and XW wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

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Supplementary material

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