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REVIEW

BIOTECHNOLOGY AND BIOENGINEERING WILEY

Ligand conjugated lipid-based nanocarriers for cancer theranostics

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Abstract

Cancer is one of the major health-related issues affecting the population worldwide and subsequently accounts for the second-largest death. Genetic and epigenetic modifications in oncogenes or tumor suppressor genes affect the regulatory systems that lead to the initiation and progression of cancer. Conventional methods, including chemotherapy/radiotherapy/appropriate combinational therapy and surgery, are being widely used for theranostics of cancer patients. Surgery is useful in treating localized tumors, but it is ineffective in treating metastatic tumors, which spread to other organs and result in a high recurrence rate and death. Also, the therapeutic application of free drugs is related to substantial issues such as poor absorption, solubility, bioavailability, high degradation rate, short shelf-life, and low therapeutic index. Therefore, these issues can be sorted out using nano lipid-based carriers (NLBCs) as promising drug delivery carriers. Still, at most, they fail to achieve site-targeted drug delivery and detection. This can be achieved by selecting a specific ligand/antibody for its cognate receptor molecule expressed on the surface of the cancer cells. In this review, we have mainly discussed the various types of ligands used to decorate NLBCs. A list of the ligands used to design nanocarriers to target malignant cells has been extensively undertaken. The approved ligand-decorated lipid-based nanomedicines with their clinical status have been explained in tabulated form to provide a wider scope to the readers regarding ligand-coupled NLBCs.

KEYWORDS

cancer theranostics, commercialization, lipid conjugates, nanocarriers

1 | INTRODUCTION

Cancer is an unregulated and uncontrolled cell division that emerges due to genetic or epigenetic changes which alter the normal signaling pathway (Akhtar et al., 2018, Choudhary et al., 2016). Whereas cancer metastasis is a series of events that result in the production of secondary tumors in distant organs and is mostly responsible for cancer mortality and morbidity. It has been reported as the second leading cause of mortality among other diseases, and incidences and fatality rates are expected to rise drastically in the coming years. At present, chemotherapy, radiotherapy, and surgery are the only alternatives to treat cancer, depending upon the nature and position of the tumor. These treatments are only limited to localized tumors and not metastatic tumors resulting in the normal cells getting affected, leading to a high recurrence rate and death. Chemotherapy, utilizing strong chemicals, has unfavorable effects on healthy cells and tissues due to a lack of specificity and cannot differentiate between normal and cancerous cells (Din et al., 2017). Also, drugs

alone cause substantial issues such as poor absorption, solubility, bioavailability, high degradation rate, short shelf-life, and low therapeutic index (Saha et al., 2016). Multiple drug resistance (MDR) has emerged as a key stumbling block in cancer treatment in recent years. As a result, developing possible therapeutic ways to treat a tumor properly is critical.

At the beginning of the 21st century, nano lipid-based carriers (NLBCs) have taken advantage of conventional targeting for therapeutic delivery in better biocompatibility, intrinsic penetrating capacity, easy fabrication, and nontoxicity (Ozpolat et al., 2014). The various attributes associated with NLBCs include drug protection, enhanced bioavailability, reduced doses, improved treatment, enhanced oral bioavailability, and surface modification flexibility. Additionally, NLBCs can precisely transport both hydrophilic and hydrophobic medicines, resulting in improved therapeutic efficiency (Ozpolat et al., 2014). NLBCs have a high surface area that can be modified with various chemical moieties such as polyethylene glycol, maleimide, poly-amino acid, carbohydrates, and fatty acids to surpass the reticuloendothelial system. Moreover, they provide platforms for conjugating the targeted ligand through various chemical reactions, including EDC-NHS cross-linking for specific receptor detection and diagnosis (Chandra et al., 2012, Mahapatra & Chandra, 2020, Pallela et al., 2016, Suman & Chandra, 2021). Incorporating bioactive compounds into comparatively biocompatible inert carriers and site-specific targeting using specific ligands represents a promising strategy for increasing its therapeutic index while lowering the side effects (Kumar, Divya, et al., 2022; Kumar, Varshney, et al., 2022). We conducted a scientific survey related to "lipid nanocarriers and cancer theranostics" through the online database "Scopus." The survey results suggested tremendous growth in the last 10 years (Figure 1). Various approaches have been utilized to deliver therapeutic medications to tumor sites, namely active targeting, passive targeting, and triggered drug delivery using nanocarriers (Tavano & Muzzalupo, 2016). The passive targeting is carried out through a perforated blood vessel system, allowing the nanocarriers to cross the membrane. Meanwhile, poorly formed lymphatic capillaries in tumor tissue limit backflow, resulting in the accumulation of nanocarriers in the tumor tissue (Maeda et al., 2013, Torchilin, 2011). On the other hand, the active targeting is facilitated by the active uptake of nanocarriers by receptor overexpressed tumor cells through their interactions with ligands (Bazak et al., 2015).

Some of the hurdles in achieving efficient drug delivery and detection through nanocarriers have been overcome; however, most issues still need to be addressed. Several surface-functionalized nanomedicines are being tested in preclinical and clinical settings, with some moving on to Phase 3 studies. Nanotechnology advancements have opened the door for developing more targeted, personalized cancer medicines. Theranostics systems that contain both a diagnostic moiety and a therapeutic agent in a single NLBCs system can track drug localization, visualization, and diseased therapy with high efficacy (Chandra et al., 2011, 2013).

In the present review, the current knowledge and individual cellular mechanism of MDR are summarized, and a detailed discussion of all the mechanisms is beyond the scope of this review.

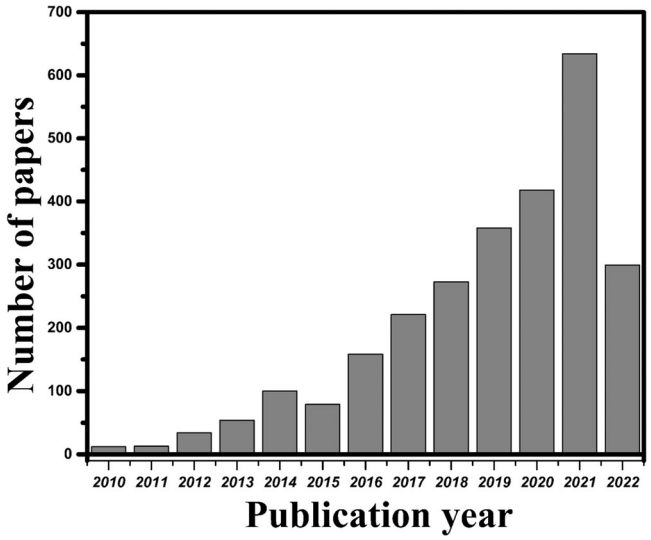

FIGURE 1 Bar-graph showing the number of research papers published each year from 2010 to 2022 in the online database "Scopus," searched using the keyword "lipid nanocarriers and cancer theranostics."

Further, considerable analysis of passive and active targeting of ligand conjugated NLBCs is well covered and discussed. We have mainly focused on the efforts to produce ligand-decorated nanocarriers, such as transferrin, folate, hyaluronic acid, aptamer, peptide, growth factor, and galactose, antibodies that have empathy for the cognate molecules on the cancer cell surface, which offer considerable potential in the theranostic of various malignancies. A list of the ligands used to adorn nanocarriers to target malignant cells specifically is included. Additionally, approved ligand-decorated NLBCs, and their clinical status have been explained in tabulated form to provide a wider scope to the readers regarding NLBCs.

2 | MDR

One of the major drawbacks that hamper the chemotherapeutic drug's potency is MDR, which can be inherited or acquired through exposure to certain chemicals. Cancerous cells utilize this and resist various substances with various action mechanisms and chemical structures. The mechanism through which MDR develops can be cellular or noncellular, accompanied by biochemical alterations and the local cellular microenvironment. Several cellular-based mechanisms, including (a) drug efflux by the ATP-binding cassette (ABC) transporter, (b) alteration in the cell cycle, (c) acidification, (d) drug encapsulation, (e) drug metabolism, (f) apoptosis inhibition, (g) nonspecific receptor binding, and (h) activation of DNA damage repair system, are illustrated in Figure 2. Nevertheless, the efflux generated by the overexpression of particular membrane transporters has been well documented and plays a significant role in MDR development. A high level of ABC transporter in MDR is the cause of efflux transport changes and reduces the drug concentrations inside

cells. The major purpose of this type of transporter is to protect cells against xenobiotics and harmful chemicals by metabolizing the drugs using ATP hydrolysis. P-glycoprotein, or ABC transporter, plays an essential role as an exporter due to its specificity for diverse substrates in cancerous cells (Bugde et al., 2017). Most of the anticancer medicines used in cancer treatment are substrates for this transporter. The tumor microenvironment, blood vessels, extracellular matrix, immune cells, and fibroblast impart a huge role in developing MDR (Yergeri et al., 2014). Many ligands conjugated lipid-based nanomedicines have taken advantage of the tumor microenvironment for detection and effective delivery of therapeutics (Cavaco et al., 2017).

3 | DRUG TARGETING

The major problems associated with free drugs include poor solubility, high degradability, and elimination in the reticulo-endothelial system. The drug must overcome numerous biological barriers to reach the target site, including cellular enzymes, cell membrane, random binding in the bloodstream, and MDR (Bhaskar et al., 2010). The barriers mentioned above can be sorted by introducing an efficient intracellular delivery system. Effective drug delivery requires a higher concentration of the drug in the body compartment, and this might cause severe toxicity or adverse side effects. Targeted drug delivery has been conceived and developed to address the lack of specificity and preserve the balance between therapeutic efficacy and toxicity (Ross et al., 2004). Various approaches, including passive targeting, active targeting, and triggered drug delivery, have been used to deliver therapeutic drugs to tumor regions using nanocarriers (Tavano & Muzzalupo, 2016). Figure 3 shows a ligand-modified nano lipid-based drug delivery approach to a tumor site.

The passive targeting took advantage of the tumor's unusual physiological features that caused the EPR effect. Angiogenesis promotes the development of abnormal blood vessels with discontinuous epithelium in tumor areas. The root of the increased permeability is the discontinuities between epithelial cells, which range in size from 100 to 800 nm and allow nanoparticles of various sizes to flow across the interstitial space (Bayón-Cordero et al., 2019). The nanocarriers can easily travel through this gap junction and reach the tumor microenvironment. Simultaneously, tumor defective lymphatic systems frequently fail to drain the extravasated nanocarriers (Maeda et al., 2013). Consequently, nanocarriers become stuck and accumulate at higher concentrations in solid tumors (Hobbs et al., 1998). Although passive targeting has been widely used in cancer therapy, it has difficulty discriminating healthy cells from malignant tissue (Steichen et al., 2013). Targeting molecules are functionalized to nanocarriers' surfaces to address these constraints, allowing them to bind to cognate molecules expressed on the cell surface actively. The nanocarriers accumulate in the tumor microenvironment before being endocytosed and transported to the cell cytoplasm (Srinivasarao et al., 2015). Many cancerous cells overexpress certain receptors on their surface (Y. H. Bae & Park, 2011). These cells overexpress certain receptors, so the specific ligand-anchored NLBCs have been widely utilized for targeted drug delivery

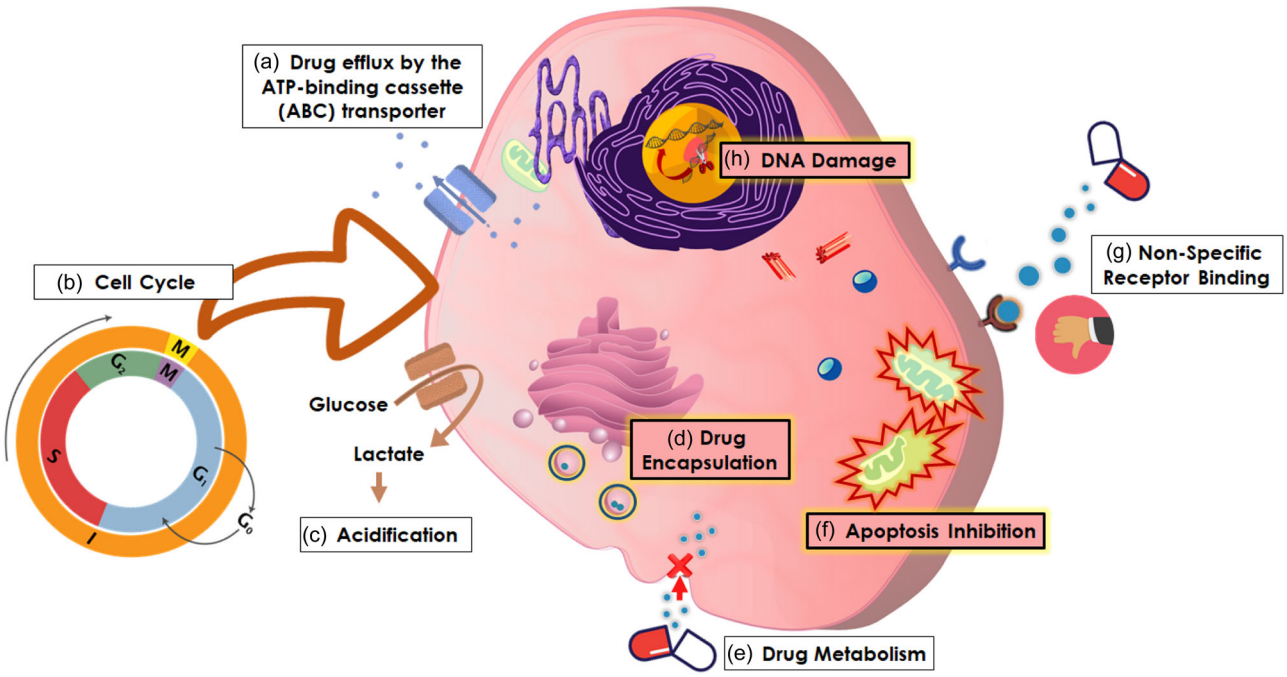

FIGURE 2 Development of multiple drug resistance in the cancerous cell using various biochemical pathways. (a) Drug efflux by the ATP-binding cassette (ABC) transporter. (b) Alteration in the cell cycle. (c) Acidification. (d) Drug encapsulation. (e) Drug metabolism. (f) Apoptosis inhibition. (g) Nonspecific receptor binding. (h) Activation of DNA damage repair system.

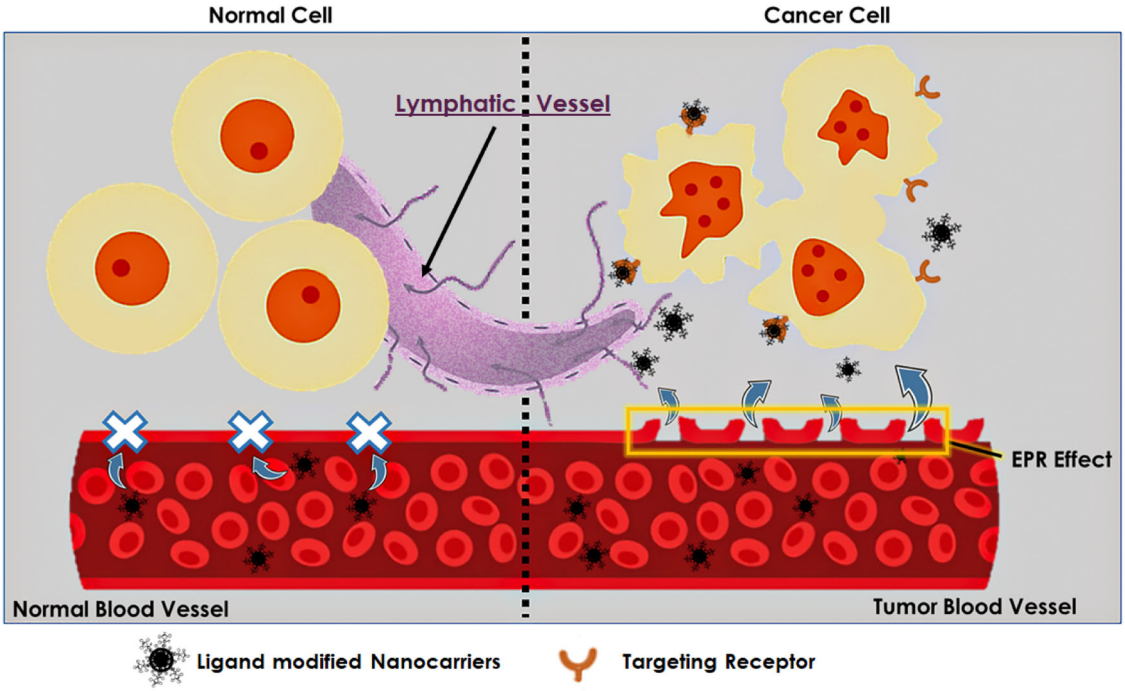

FIGURE 3 Enhanced permeability and retention in cancer tissue. Extravasation of ligand-modified nanocarriers is terminated due to continuous vascular epithelium in a normal cell. The epithelial and poor lymphatic vessel's perforated membrane allows the nanocarrier to accumulate in the cancerous cells.

and detection (Yoo et al., 2019). Many targeting ligands have been employed, including small compounds, carbohydrates, peptides, proteins, and antibodies for efficient theranostics. Additionally, modified PEG (polyethylene glycol) has been applied to the surface of nanocarriers to extend the shelf-life of drugs in the circulatory system. Moreover, the most typical benefit of surface conjugation is that it reduces nanocarrier clearance from the spleen and liver because of the tumor's disordered microenvironment and leaky vasculature system. Active targeting using nanocarrier decorated ligands has spurred cellular absorption, reduced side effects, and lessened anticancer drugs' immunogenicity (Zorko & Langel, 2005). In vitro, in vivo, and clinical studies have proven the potential of active targeting over conventional targeting.

4 | NLBCs AND TARGETING MOIETIES

The primary goal to utilize surface-expressed receptors is to successfully deliver drugs and detect malignancies using ligand-decorated NLBCs. Liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) are major types of NLBCs and can be employed in cancer treatment to achieve high diagnostic and therapeutic standards, especially in cancer patients. Liposome is formed spontaneously by polar lipid or amphiphilic molecules with a polar head group and a long hydrophobic tail, such as PEGylated phospholipids, lecithin, DSPE, and DSPC. Cholesterol helps provide stability to the liposomal structure and enhances the solubility of drugs in the blood circulation system. During the preparation of liposomes, cholesterol may form large vesicles of sizes ranging from 0.025 to 2.5 μm (Yingchoncharoen et al., 2016). However, the cells can uptake the developed liposome drug carriers either by passive or active targeting. In the case of passive targeting, the drug-loaded liposome is taken up via molecular diffusion through the cell membrane. On the other hand, active targeting is carried out by surface modification of the carriers with specific ligands. The lipids, solid at room and body temperature, such as purified triglycerides and fatty acids, are replaced with the liquid lipid (oil) component of an oil/water emulsion to create SLNs. The SLNs are constituted of 0.1% (w/w) to 30% (w/w) solid lipid dispersed and stabilized in an aqueous medium with preferably 0.5% (w/w) to 5% (w/w) surfactant. Numerous characteristic features, including high surface-to-volume ratio, non-immunogenicity, biodegradability, and solid nature at room temperature with an easy scale-up process, make SLNs a suitable drug delivery carrier (Mukherjee et al., 2009). However, SLNs have certain limitations, including limited drug loading efficiency and drug discharge during the crystallization process. The NLCs, called second-generation NLBCs, are synthesized using two monomers (Esposito et al., 2012), solid and liquid. NLCs comprise a lipid matrix with a unique nanostructure designed as a new generation of lipid nanoparticles. This kind of carrier can overcome the common problems associated with the usual carriers, such as low drug loading efficiency and drug leakage during crystallization, whereas increasing cellular uptake with higher permeability and retention effects. Nanocarrier design and formulation laid the basis for receptor-mediated cellular entrance via endocytosis (Scioli Montoto et al., 2020). The proper selection of ligands is crucial in developing

the potent carriers to selectively diagnose following preferential uptake of drugs in the cancerous cells (Asghari et al., 2017). Various types of cell surface receptors on cancerous cells, targeted using suitable targets, have been illustrated in Table 1.

4.1 | Transferrin receptor

A glycoprotein helps in iron capturing from the circulatory system and facilitates cellular internalization through receptor-mediated endocytosis. Also, it involves controlling the iron influx in cells, thereby sustaining homeostasis. The receptor is a prospective target site for specific ligands and antibodies because of elevated levels of its expression, which can be 100-fold greater than normal cell expression (Daniels et al., 2006). The fundamentals of chemistry play a key role in conjugating specific ligands or antibodies with the surface of NLBC for precise, controlled, and site-directed delivery of anticancer drugs with selective detection. Aqueous stability testing in a biologically simulated environment reveals that the transferrin-conjugated NLBCs (Tf-NLBCs) are more stable and facilitate drug release in a targeted manner. Figure 4a shows the step-by-step synthesis of transferrin conjugated NLBCs, and the mouse xenograft model determines the biodistribution of the formulation. The structural components of lipid carriers are soya lecithin, oleic acid, tween 80, glyceryl monostearate, and PEG-PE, which provide strong stability and high encapsulation efficiency. The fate of the structure of the NLBCs is dependent on the types of lipid monomer used during the preparation of the carrier. The PEG-PE provides the PEG length and terminal moieties, making the covalent bond with the targeted Tf receptor. It was observed that the efficacy of Tf-decorated NLBCs enhanced 10-fold as compared to free drugs. In the context of anticancer drug delivery to specific sites, numerous researchers constructed and evaluated the efficacy of Tf-NLBCs in diverse cancer cell line and mouse models. In another study, X. Li et al. (2009) successfully utilized the overexpressed Tf receptor on the malignant cell surface for designing the nanocarrier. They constructed Tf-coupled liposomal vesicles for targeting doxorubicin as a potent anticancer drug. Film dispersion and ammonium sulfate gradient methods were employed to synthesize liposomal vesicles. Tf was functionalized on the cell surface of the vesicles comprising DSPE-PEG_{2000}-COOH through EDC−NHS crosslinking. The developed construct was evaluated against cancerous cells, and it was found that the Tf-coupled liposomal vesicles accumulate more drugs inside the cells than uncoupled vesicles. However, the cytotoxic effect induced by the developed construct on normal cells was minimal. In addition, pharmacokinetics studies in tumor mice revealed that coupled vesicles effectively delivered the drugs to the targeted tissues; however, the concentration of the drugs was found to be minimum in nontargeted tissue such as the heart and kidney. The weight of the Tf conjugated doxorubicin-liposome-treated tumor was observed to be 0.33 g, whereas the weight of the tumor treated with only PEG-liposome-doxorubicin and free doxorubicin was found to be 1.17 and 1.38 g, respectively (X. Li et al., 2009). Muthu et al. (2015) developed a theranostic micelle composed of D-alpha-tocopheryl polyethylene glycol succinate conjugated with Tf for the specific administration of docetaxel (therapeutic agent) and gold clusters (bioimaging module). A casting method was used to synthesize micelles, and then Tf was coupled to the surface of the micelles through carbodiimide chemistry. The formulated construct was tested in Tf overexpressed MDA-MB-231 cells and NIH-3T3 fibroblast cell line (no Tf overexpression). The flow cytometry study showed that the intensity of fluorescein (FITC)-stained MDA-MB-231 cells significantly shifted after being treated with formulated micelle. However, no significant shift was observed in the NIH-3T3 cells (FITC staining) treated with the same inducers. Further, formulated micelle showed a fourfold reduction in IC_{50}value in MDA-MB-231 cells compared to NIH-3T3 cells. It is also observed that gold clusters showed a strong fluorescence signal with photostability property in malignant cell imaging (Muthu et al., 2015). The Tf and NLBCs allow for improved therapeutic efficacy, prolonged circulation, and a better release profile, preventing nonspecific binding with increased toxicity of the free drugs at the targeted site. However, certain drawbacks are observed while using a lipid system conjugated with Tf. Drug encapsulation and loading efficiency of Tf conjugated NLBCs were found to be decreased as compared to nonconjugated nanocarrier. In addition to this, a long chain of PEG-polymer may not detach from the nanocarrier in the tumor microenvironment. Another drawback of employing Tf conjugated NLBCs is that the protein corona effect on Tf-NLCs may adversely affect Tf receptor targeting, thus slowing Tf -dependent absorption in the targeted region (Pitek et al., 2012). Also, high endogenous Tf levels in plasma, which saturate the Tf receptors in the blood−brain barrier, are another limiting factor in Tf-NLC accumulation in the brain. Because Tf-nanoparticle competes with endogenous Tf for receptor binding, the amount of Tf conjugated nanoparticle accumulation in the brain will be reduced (Meng et al., 2018). Overall, the novel nanomedicines based on lipid and transferrin offer promising combinational cancer theranostics.

4.2 | Folate receptor

Folic acid is water-soluble, naturally found in many foods, and a key component in the production of nucleic acid. Folate has a high affinity to folate receptors (FR), expressed in 40% of human carcinoma, facilitating drug uptake by receptor-mediated endocytosis (Low & Kularatne, 2009). Folate acid (FA) targeted drug delivery can be achieved by designing suitable linkers for specifically targeted cells to accomplish cytotoxic activities. Folic acid is a tiny substance with a 440 Da molecular weight stable over a diverse pH and temperature range. It is nonimmunogenic and facilitates drug accumulation after conjugating with antitumor drugs or testing markers. The cells internalize the conjugated carrier through endocytosis, a noninvasive design for imaging cancer cells. The folic acid conjugated drug-loaded micelle and liposome with their internalization in tumor-bearing mice is well represented in Figure 4b. Egg phosphatidylcholine, cholesterol,

TABLE 1 List of ligands and their target molecules with nanocarriers

S. no.	Ligand	Targets	Nanocarriers	Tumor	References
1	RGDF peptides	Integrin receptors ($\alpha v\beta_3$, $\alpha v\beta_5$)	Micelles	Murine hepatic carcinoma (H22 cell)	Liu et al. (2018)
2	A54 peptide	Hepatocarcinoma cell	NLCs	Hepatic tumor (Bel-7402 cell)	Lu et al. (2017)
3	cRGD	Integrin receptors ($\alpha v\beta_3$)	Micelles	Astrocytoma (U87 cells)	X. Chen et al. (2017)
4	cRGD	Integrin receptors ($\alpha v\beta_3$)	Micelles	Brain tumor (U87MG cell)	Quader et al. (2017)
5	Lyp-1 peptide	Tumor site	Micelles	Breast cancer (4T1 cell)	W. Li et al. (2016)
6	hEGF ligands	EGFR	Liposome-like nanovesicles	Ductal carcinoma of the breast (BT474 cell)	P. Zhang, Zhang et al. (2018)
7	Hexapeptide ligand AE	EGFR	Micelles	Astrocytoma (U87 cell)	Mao et al. (2017)
8	Anti-CD44 antibody	EGFR	Micelles	Breast tumor (MCF7 cell)	Gener et al. (2015)
9	HIV trans-activating transcriptional activator (TAT) peptide	Nuclear pore complexes	Micelles	Cervical cancer (HeLa cell)	Han et al. (2015)
10	EGa1 nanobodies	EGFR	Micelles	Squamous cell carcinoma (14C cell)	Talelli et al. (2013)
11	Hyaluronic Acid	CD 44	SLNs	Melanoma cancer (B16F10 cell)	Shen et al. (2015)
12	Galactose	Lectin receptors	SLNs	Adenocarcinoma (A549 cells)	Jain et al. (2015)
13	Transferrin	Tf receptors	NLCs	Human non-small cell lung carcinoma cell line (NCI-H460 cells)	Shao et al. (2015)
14	Folate	Folate receptor	Liposome-PLGA nanoparticles	Epidermal carcinoma cell line, K.B. cells, lung carcinoma cell line, A549 cells	S. H. Kim et al. (2005)

Abbreviations: EGFR, epidermal growth factor receptor; NLC, nanostructured lipid carrier; SLN, solid lipid nanoparticle.

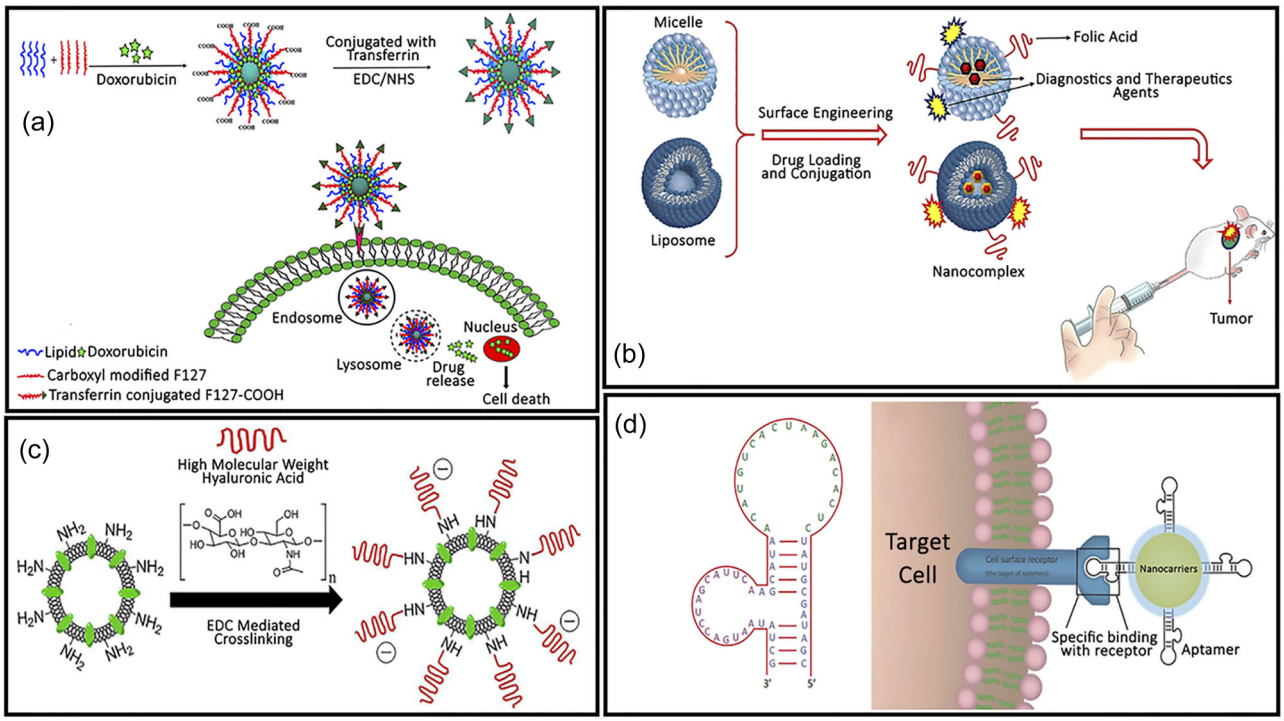

FIGURE 4 Schematic representation of ligand conjugated nanocarriers. (a) Synthesis of Tf conjugated nanocarriers and its interactions with cognate molecule. Reproduced with permission from Soe et al. (2019). (b) The FA conjugated drug-loaded micelle and liposome and their internalization in tumor-bearing mice. Reproduced with permission from Narmani et al. (2019). (c) EDC-mediated crosslinking of HA on the surface of lipid nanocarriers. Reproduced with permission from Hayward et al. (2016). (d) RNA-based aptamer and aptamer-decorated nanocarrier and its interaction with the cognate receptor. Reproduced with permission from He et al. (2020). EDC, ethyl-3-(3-dimethylamine propyl) carbodiimide; FA, folate acid; HA, hyaluronic acid; Tf, transferrin.

cholesteryl hemisuccinate, and DOPE are the main structural components of NLBCs (liposomes and micelles). The structural components of the liposome align in a spherical structure that has been formed either by a single lipid bilayer or multi-lipid bilayer when mixed with an aqueous solution. However, micelles are simple aggregates of lipids with a hydrophobic core. The outer surface of liposomes/micelles exposed with DOPE is exploited for conjugation with FA through covalent bonding. The FA-targeted liposomes/micelles demonstrated a 2.20-fold increase in cell toxicity compared to free drugs (Luong et al., 2017). In various investigations, NLBCs were used as a linker for conjugating FA and anticancer drugs. Pawar et al. (2016) synthesized FA-decorated SLNs (FA-SLNs) using an emulsification technique for tumor-specific delivery and targeting docetaxel and curcumin. Glyceryl monostearate, compritol 888 ATO, poloxamer 188, and PEG-stearic acid are the main structural components of the SLNs. In general, while preparing the SLNs, oil is replaced by solid lipids (phospholipid (90 NG)/Glycerol tristearate/Glycerol monostearate/Cetyl palmitate) in the emulsion, which improves the encapsulation efficiency stability of the system. The EDC–NHS chemistry is used to conjugate FA to PEG-stearic acid. Formulation parameters such as stabilizer concentration, stirring speed, homogenization time, lipid concentration, stabilizer composition, and types of lipids used were optimized through a central composite design. The obtained nano construct was attributed to high drug encapsulation and desirable particle size with spherical morphology., Moreover, cellular uptake and tissue distribution studies were conducted on MCF-7 cells and Wistar rats. Fluorescence microscopy revealed that the FA-SLNs accumulated 36.84% more drugs compared to unconjugated SLNs inside the cells with minimum toxicity. Further, methotrexate at the dose of 15 µg/ml, when encapsulated in the FA-SLNs, induced toxicity to cancer cells and spared the normal cells. However, tissue distribution studies showed that docetaxel accumulation was maximum in the liver (Pawar et al., 2016). Yassemi et al. (2020) similarly utilized the FR for tissue-specific delivery of letrozole through FA-SLNs. First, SLNs were synthesized using structural components of tripalmitin glyceride, octadecyl amine, and the surfactant, Tween-80, by a solvent emulsification evaporation method. After that, the folate was conjugated with the amino group functionalized carrier through carbodiimide chemistry. The obtained nanocarriers were spherical with high encapsulation efficiency, and the drugs were dispersed into the amorphous structure. Moreover, the formulated nano construct was evaluated on MCF-7 to see the effect of cell toxicity. Results suggested that the efficacy in terms of IC50 of FA-SLNs was found to be 2.85- and 11-fold greater than unconjugated SLNs and free drugs, respectively (Yassemi et al., 2020). The combination of FA and NLBCs, cytotoxicity, and selective permeability of NLBCs to pass through endosomal membrane indicates FA-NLBCs to be a powerful

construct in the therapy and detection of various malignancies. Tumor cells overexpressed FR, whereas healthy cells expressed less folate carrier (RFC). The binding affinity of FA to RFC is considerably weaker than the binding affinity between FA and FR. As a result, an FA-conjugated polymer was used, which had a higher affinity for cancer cells than normal cells. In comparison to Tf-mediated drug targeting, FA induces a more cytotoxic effect because FA directly targets the drug at the site of nucleotide synthesis, such as the nucleus. However, the presence of folic acid on the surface of the lipid carrier decreases the overall negative charge of the carrier, which further influences the cellular uptake and cytotoxicity of the free drugs and also affects the stability of the carrier. The main challenges associated with FA-decorated nanocarrier are assessing pharmacodynamic and pharmacokinetic facts, evaluating its interactions with biomolecules in the body serum, biodistribution, and determination of subcellular and cytotoxic behaviors. On the other hand, the concrete applications of FA-decorated nanocarriers in the human system are poorly understood. As a result, comprehensive research involving clinical trials in diverse cancer types is required to learn more about the efficacy of FA conjugated nanocarriers in cancer detection and therapy.

4.3 | Hyaluronic acid

It is a polysaccharide consisting of repeating units of disaccharide *N*-acetyl-D-glucosamine and D-glucuronic acid through (β1 ⟶ 3) bonds. Around 2000 repeating disaccharides are joined together by a (β1 ⟶ 4) glycosidic bond to form a massive polysaccharide. This molecule plays a huge role in migration, angiogenesis, proliferation, differentiation, and invasion by binding with specific receptors, for example, CD44. Hyaluronic acid (HA) is a primary structural element of the cellular matrix and is considered an efficient drug delivery material (Dostalek et al., 2013). Malignant cells such as pancreatic cancer, lung cancer, breast cancer, ovarian cancer, and prostate cancer overexpress the CD44 receptor on their cell surface (Coradini & Daidone, 2004). It is a multifunctional transmembrane glycoprotein that plays an important role in tumor motility, self-renewal, and drug resistance and prevents apoptosis in cancer cells. More interestingly, CD44 is linked to self-renewal cancer stem cells; therefore, targeting it with a specific ligand–drug conjugate may destroy the cell population and eradicate cancer (Mimeault & Batra, 2010). To achieve a dual role, such as tumor detection and removal of cancer stem cell-rich populations, targeting CD44 is a viable option for generating more successful cancer theranostics. The HA modulates the tumor microenvironment and subsequently promotes a malignant phenotype via interaction with the CD44 receptor, further inducing the intracellular signaling pathway (W. Wu, Chen, et al., 2020). The coupling of the HA to the surface of the NLBCs has the following advantages. First, to begin with, HA on the outer shell of particles protects carriers while also regulating circulation time and bioavailability. Second, as the main structural element of the extracellular matrix, HA is biocompatible and prevents the nanocarriers from nonspecific bindings with high efficiency. Figure 4c shows the amino expressed NLBCs (liposomes) and their coupling with HA through EDC–NHS cross-linking (Mimeault & Batra, 2010). L-α phosphatidyl choline, DPPE, and cholesterol are the main structural components used to synthesize the liposome nanocarriers. The DPPE comprising the primary amine group has been utilized for preferential conjugation with the carboxylic group of the HA through amide bond formation. The HA conjugated liposomes showed 4.48-fold more efficiency on CD44 expressed cancer cells compared to normal cells. Shen et al. (2015) have successfully utilized CD44 expressing cancer stem cells to design HA conjugated SLNs (HA-SLNs). Further, they tested the HA-SLNs for paclitaxel delivery to the CD44 expressing melanoma cells and mouse xenograft model. They found that the HA-SLNs induced apoptosis in the CD44 expressing cells and efficiently delivered the paclitaxel into the melanoma lung tissue (Shen et al., 2015). In an investigation, glioblastoma multiforme (GBM), brain malignancy cell has been effectively targeted using HA-decorated liposome. GBM cells that overexpressed CD44 have effectively accumulated doxorubicin, used as a potential anticancer agent compared to normal cells. The HA coupled liposome was tested against nonmalignant cells (primary cortical astrocytes and primary microglia) and malignant cells (A-172). The comparative study found that HA conjugated liposome promoted preferential uptake of anticancer drug to A-172 cells with lethal concentration (LC_{50}) of 0.114 ± 0.010 as compared to primary cortical astrocytes, LC_{50} of 0.511 ± 0.039 and primary microglia, LC_{50} of 0.317 ± 0.048. The aforementioned results suggested that HA conjugated liposomes selectively diagnosed and accumulated the drugs in the malignant cell (Hayward et al., 2016). In another fascinating study, stem cells with a high level of CD44 expression were successfully used to build an effective drug–nanocarrier construct. Initially, a delivery system based on stem cells that expressed strong phenotypes such as cancer stem cell-related marker, colony formation, and tumor outgrowth in vivo has been successfully developed. Further, the delivery system of hyaluronic acid conjugated paclitaxel-loaded solid lipid nanoparticles (HA-SLNs/PTX) has been synthesized and tested. Glyceryl monostearate, soy phosphatidyl choline, and cholesterol are the main structural components of the SLNs. The electrostatic attraction approach was used to fabricate the HA-SLNs/PTX system. Dose-dependent cytotoxicity of HA-SLNs/PTX and SLNs/PTX was assayed on B16F10-CD44+ and A549 cells. The IC50 value of HA-SLNs/PTX is (11.13 ± 1.62 μg/ml), followed by SLNs/PTX (18.11 ± 3.79 μg/ml) and then PTX (free) (31.39 ± 4.81 μg/ml) when these inducers were tested on the B16F10-CD44+ cells. Similar outcomes were obtained in A549 cells, along with the IC50 value 23.99, 28.90, and 40.89 2.72 μg/ml, induced with HA-SLNs/PTX, SLNs/PTX, and PTX (free), respectively. The obtained values suggest HA-SLNs/PTX detects carcinogenic cell lines effectively and shows greater efficacy in terms of anticancer activity (Shen et al., 2015). Overall, conjugating HA with NLBCs surface could be a practicable approach for detection and anticancer drug delivery. Moreover, circulation time, bioavailability, and enzymatic degradation of the anticancer drug can be enhanced significantly using HA-mediated

lipid formulations. It is also noticed that by selecting high molecular weight HA, the overall charge of the carrier remains unaffected and preferentially accumulated the drugs at the targeted site. Further, the HA conjugated lipid nanocarrier when administered in blood circulation of the body, the conjugate swells up and creates a cloud of a long chain of HA, which protects the carrier from opsonization from the macrophage. The expression of genes involved in proliferation and inflammation is not induced by HA, proving the effective "bioinert" component of drug delivery systems. However, the density of the HA on the surface of the lipid carrier is one of the important parameters which needs to be optimized since higher density increases the aggregation of NLBCs. However, long-term stability and clinical translations are major drawbacks of the HA conjugated NLBCs.

4.4 | Aptamer

Single-stranded deoxyribose or ribonucleic acid-based oligonucleotide is synthesized to bind with cognate molecules expressed in various types of melanoma cells. Different properties such as small size, tissue-specific penetration, high binding affinity, nonimmunogenicity, and amenable modification make the aptamer a promising theranostics linker (Wandtke et al., 2015). Once binding between the aptamer and cognate molecule is achieved, the nanocarriers enter the cells through the plasma membrane. The aptamer may form various secondary structures because it makes self-complementary base pairs. The secondary structure of aptamer can arrange itself in a three-dimensional structure that further interacts with cell surface receptors through various weak bonds such as electrostatic attractions, van der Waals forces, π–π stacking, hydrophobic interactions, or even structure match (Zhou & Rossi, 2017). Different types of chemical modifications such as the replacement of phosphorodithioate and 2^1–o methyl in one nucleotide, replacement of phosphodiester backbone with boranophosphate of 2^1–OH methoxy motif phosphothoate, reactive 2^1–OH base of RNA functional group, incorporation of fluoro, 2^1–OH amino protect the aptamer from cellular enzymatic degradation. Moreover, the nonbridging oxygen atom is substituted by one or more sulfur atoms which further enhances the stability of the aptamer (Volk & Lokesh, 2017). On the other hand, DNA-based aptamer is highly recommended since it is resistant to 2^1 endonucleases. An S1411 is a 26-mer G-rich DNA oligonucleotide specific for a cognate molecule, nucleolin, overexpressed in various tumor cell types. The aptamer is useful in nanotechnology, neurosciences, medical imaging, and cancer-targeted therapy because of its exceptional qualities (Röthlisberger et al., 2017). NLBCs modified with aptamer play a huge role in cancer theranostics in this direction. During the preparation of various structural forms of NLBCs, an aptamer can be coupled with a lipid tail forming a three-dimensional structure. Multiple studies were reported using aptamer as a linker and NLBCs as a drug delivery carrier for successful theranostics of cancerous cells. The aptamer-decorated liposomal nanocarrier and its interaction with the receptor is well illustrated and presented in Figure 4d. The EPOPC, cholesterol, rhodamine-DOPE, and DSPE-PEG2000-maleimide are the monomers used in the preparation of liposome nanocarrier using the lipid hydration method. Tris (2-carboxyethyl) phosphine hydrochloride is an efficient reducing agent used to reduce the AraHH001 aptamer and expose 5'-thiol. Through thiol reaction, the 5'-thiol-modified aptamer was further conjugated with C1 carbon of the maleimide functionalized on DSPE-PEG2000. The drug accumulation efficiency using aptamer conjugated liposome was almost 3.8-fold higher than that of PEG-liposome system, which suggested the potent detection of biomarkers (Ara et al., 2014). Kang et al. successfully constructed an aptamer conjugated lipid micelle-based nanocarrier to detect epidermal growth factors expressed on cancerous cells and deliver an anticancer drug, paclitaxel. The DSPE-PEG_{2000}-maleimide and methoxy (PEG-2000)-DSPE are the main structural component of the micelle. However, paclitaxel and quantum dots (QDs) are encapsulated in the micelle as anticancer drugs and optical imaging agents, respectively. The DSPE-PEG_{2000}-maleimide was anchored on the surface of the micelle, which provides the linker through which antibody and reduced aptamer were conjugated successfully. Immunomicelle and aptamo-micelle have tumor-targeting capacities similar to each other and have an efficiency roughly fivefold higher than nontargeted micelle (Kang et al., 2018). Alshaer et al. (2015) developed the 2^1-F pyrimidine-containing RNA aptamer functionalized liposome for selective targeting of CD44 expressed tumor cells. The structural components of the liposome are DPPC, cholesterol, and DSPE-PEG-maleimide. Thiol-maleimide conjugation chemistry was used to conjugate 3'-thiol-modified Apt 1 with maleimide functionalized to the surface of the liposome. Further, the intracellular distribution of the construct was evaluated using confocal microscopy for lung cancer cells and mouse embryonic fibroblast cells. Results suggested that the aptamer-liposome selectivity distributed the drugs compared to free liposome in tumor cells. Further, when A549 and MDA-MB-231 cells were treated with Apt1 conjugated liposome instead of unconjugated liposome, the mean fluorescence intensity of both the cells increased significantly. There was no significant change in mean fluorescence intensity between Apt1 conjugated liposome and unconjugated liposome with NIH/3T3 negative cells (Alshaer et al., 2015). In another interesting study, non-small cell lung cancer (NSCLC) was targeted using an aptamer conjugated lipid-polymer hybrid system (ALPHS). The hybrid system was synthesized using glyceryl monostearate, thiodiglycolic anhydride, lecithin, Poly(L-lactide) (5000)-poly(ethylene glycol) (2000)-maleimide (PLA-PEG-MAL) by a one-step precipitation method and loaded with docetaxel (DTX) and cisplatin (DDP), a potential anticancer drug. A549 cell-binding aptamer (S6, sequence: GTGGCCAGTC ACTC AATTGGGTGTAGGGGTGGGGATTGTGGGTTG) with a sulfhydryl group was conjugated with PLA-PEG-MAL component through thiol reaction. The release kinetic pattern of the constructed hybrid system is significantly improved, which facilitated the sustained rate of drug release. DTX/DDP-ALPHNs had significantly improved cytotoxicity by 50%, and tumor inhibition ability by 81.4% as compared to non-ALPHNs and single drug-loaded LPHNs (R. Wu, Zhang, et al., 2020). This selectivity of aptamer was found to be 10-fold higher than that of the antibody and they first recognize the cell surface and then facilitate

cellular internalization. However, serum stability, renal filtration, and endocytic escape are all typical limitations of using aptamer as a ligand. Other disadvantages include a lack of diversity in the aptamer library, vulnerability to nucleases, and quick degradation in blood circulation. Further, the long chain of PEG-polymer causes the steric hindrance on the nanocarrier surface, which reduces the cellular-based uptake of the drugs. Despite several efforts for producing efficient aptamer-based nanomedicine, only a small percentage of nanomedicines have been successfully utilized in clinical applications. Therefore, aptamer–drug –NLBCs techniques should be expanded, and in this direction smart or environment-responsive linkers could help in detecting and accumulating the drugs in précised manner.

4.5 | Peptide

The peptide is a chain of oligo amino acids. The carboxylic group of one amino acid makes a covalent bond with an amino group of another and has great potential in the field of theranostics. Presently, the market value of peptide-based nanomedicines is presumed to be US$40 billion/year of the total pharmaceutical market (Craik et al., 2013). The continuous emerging medicines market will contrive an even larger contributor shortly. Although small molecule-based nanomedicine is presently the market's largest contributor, peptide-based nanomedicine has emerged with greater specificity because of multiple contact points with the cognate's receptors. Therefore, many researchers have engineered NLBCs using peptides as a linker for anticancer drug delivery and detection of cancer cells as well. Many peptides, such as LyP-1, K237, RGD (arginine–glycine–aspartic acid), and bombesin, are employed to adorn the NLBC surface to direct the overexpressed biomarkers on various tumor progressions such as melanoma, breast, and ovarian cancer, and so on (J.-H. Kim et al., 2012, Venkatalaxmi et al., 2004, Yu et al., 2010). The RGD peptides are well recognized for their ability to function as a strong recognition motif for a variety of integrins, such as αvβ3 and α5β1, found on cancer cell surfaces. These surface receptors are over-expressed in the tumor cells, and therefore, RGD peptide is used as a linker in numerous engineered NLBCs for cancerous theranostics. Xiong et al. developed a stabilized RGD-coupled liposomal vesicle (RGD-LV) for doxorubicin delivery and compared its performance with liposomal doxorubicin and free doxorubicin in vitro and in vivo conditions. Flow cytometry study demonstrated that RGD-decorated liposome induced more drug accumulation with comparatively enhanced intracellular uptake in B16 and A375 cells. Yang et al. simultaneously examined the cumulative effect of two anticancer drugs, doxorubicin and 5-fluorouracil in PC-3 human prostate carcinoma. They used a G3-C12 linker to modify the surface of an acrylamide polymer nanosystem to anchor doxorubicin and 5-fluorouracil. In this system, hydrazone, a pH-responsive connector, was used to bind doxorubicin to the covalent nanocarriers. However, 5-fluorouracils were conjugated to the NH_2-PEG_{2000}-DSPE using oligopeptide. The constructed nanocarriers showed high cytotoxicity with significant drug accumulation in prostate carcinoma overexpressed galectin. Additionally, the formulation increases cytotoxicity because of drugs' synergistic genotoxicity, caspase-3 activation, cell cycle arrest, and DNA damage, resulting in tumor progression inhibition in in vivo system as compared to nontargeted nanocarriers (Yang, Li, et al., 2014). Cell-penetrating peptides (CPPs) are a type of cationic amino acid-based oligopeptides that can cross cell membranes and deliver cargo in a precise manner. Although the CPPs are not specific for any receptor, they are widely utilized as a linker to engineer the NLBCs in numerous malignancies. Poly-arginine is a cell-diffusing oligo-peptide with an optimal chain length of 8 arginine units that have been successfully employed to deliver anticancer drugs intracellularly. Figure 5a shows the design of a thermosensitive liposome (TSL) containing CPP-doxorubicin (CPP-DOX) conjugate, and its surface is decorated with NGR (Asparagine-Glycine-Arginine) for detection and delivery of doxorubicin to aminopeptidase N(APN/CD13) overexpressed cancer cells. The MSPC, DPPC, methoxy (PEG-2000)-DSPE, DSPE-PEG2000-Mal) are the main structural components of the TSL. As the primary targeting and detecting moiety for the specific tumor cells, NGR was conjugated to the exposed terminal of the PEG chains on the surface of the TSL(NGR-TSL). However, CPP was conjugated with doxorubicin using EDC–NHS and employed as secondary targeting moiety encapsulated in TSL to enhance the biodistribution of the drugs. The encapsulation of CPP inside the TSL protects the peptide from enzymatic degradation in the blood circulation. The presence of PEG-chain on the liposomal surface facilitates the carrier to be transported in the tumor environment and subsequently enhanced the permeability retention (EPR) effect. The size and the zeta potential of the final nano bioconjugate were increased because of peptide addition which shielded the negative charge produced due to the phosphate group from the liposome. The efficacy in terms of cell toxicity of CPP-DOX/NGR-TSL was 1.5-fold higher than CPP-DOX/TSL; however, no significant results were observed in normal cells (Yang, Yang, et al., 2014). Biswas et al. engineered pegylated liposomes using CPP R8 (octa-arginine) with doxorubicin as a potential anticancer drug. methoxy (PEG-2000)-DSPE, PEG2000-DOPE provided the moieties through which R8 was conjugated to form R8 modified liposome. The developed formulation was tested in A549 (lung cancer cell line) to examine the bioavailability and biodistribution of drugs in a target-specific manner. They observed that the cell death marker, lactate dehydrogenase, increased significantly with the above formulation comprising doxorubicin. At the Dox concentration of 100 μg/ml, R8-modified liposome therapy revealed a 55% drop in cell viability compared to 10% with liposome. In addition, the percentage cell viability for R8-liposomal treatment was found to be 45.35 ± 2.14%, compared to 89.6 ± 5.9% for liposome alone at 100 μg/ml of Dox. In the A549 tumor, the expression of caspase 3/7 elevated significantly, further inducing apoptosis by ninefold, and reduced the tumor volume with tumor weight after treatment with R8-liposome. The proposed formulations specifically detected the receptor and enhanced doxorubicin delivery effectively in a cancer model system (Biswas et al., 2013). In one of the interesting studies, a peptide HVGGSSV was isolated from an in vivo screening of phage-displayed

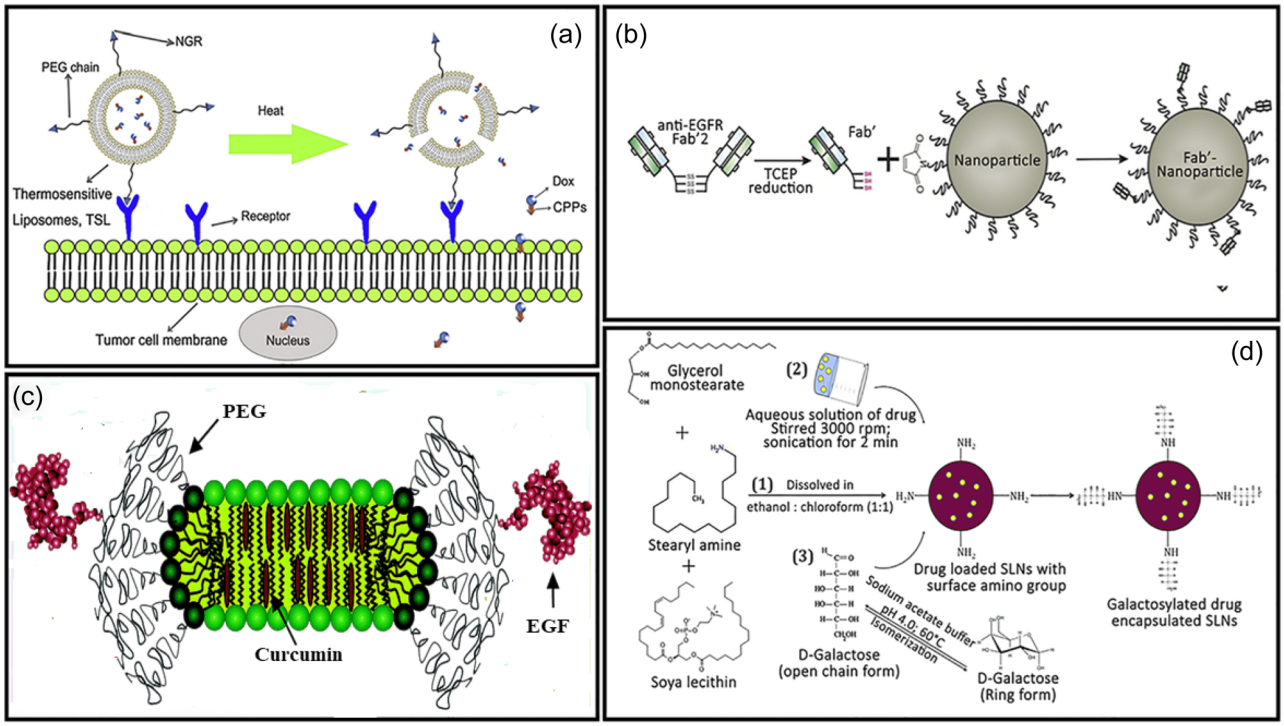

FIGURE 5 Schematic illustration of ligand conjugated nanocarriers. (a) NGR- conjugated thermosensitive liposome containing CPPs-DOX for detection of receptor and enhancing the DOX biodistribution. Reproduced with permission from Sun et al. (2016). (b) Schematic representation of the synthesis of Fab' conjugated nanoparticle. Initially, reduction of anti-EGFR Fab' 2 to Fab' fragment through Tris/2-carboxyethyl phosphine hydrochloride (TCEP) and generating three active thiol groups (-SH), which further react with maleimide group expressed on the nanoparticle surface. Reproduced with permission from Zhai et al. (2015). (c) EGF expressed nanocarrier for cancer theranostics of curcumin and doxorubicin in A-431 tumor cells. Reproduced with permission from Ahlgren et al. (2017). Step-by-step synthesis of galactosylated conjugated drug-loaded SLNs. Initially, ring opening of galactose molecule and further cross-linked with amino group of stearyl amine exposed on the surface of SLNs through the EDC/NHS bioconjugate chemistry. Reproduced with permission from Jain et al. (2015). CPP-DOX, cell-penetrating peptide-doxorubicin; EGF, endothelial growth factor; SLN, solid lipid nanoparticle.

peptide library due to its selective binding within irradiated tumors and engineered on the surface of the doxorubicin-loaded liposome since it had a high affinity for lung carcinoma. The thin-film hydration technique was employed for the synthesis of liposomes using structural components of cholesterol, DSPC and maleimide-PEG2000-DSPE. The cysteine-containing peptide was conjugated with liposomal surface modified with maleimide. Near-infrared imaging was used to examine the biodistribution of the peptide-modified liposome labeled with Alexa Fluor 750 within the irradiated lewis lung carcinoma. The doxorubicin-targeted liposome exhibited a long circulatory half-life and increased doxorubicin deposition within tumors to the point where it lasted more than 20 h after intravenous delivery. Also, the formulated liposome accumulated the doxorubicin level twofold higher as compared to free doxorubicin. Moreover, modified liposomes reduced the tumor growth, enhanced blood and lymphatic vessel destruction, and increased apoptosis in a mouse bearing carcinoma (Lowery et al., 2011). Peptides as potential ligands are attracting a lot of attention among active ligands because of their unique advantages, such as ease of preparation, low cost, and high potency. However, for the time being, utilizing peptides as active targeting ligands has some drawbacks. Peptide ligands typically have a shorter half-life, which could result in premature degradation and dissociation of the payload before it reaches the target site. Meanwhile, peptide ligands have lower binding affinity and selectivity than antibodies or proteins, which enhances the likelihood of off-targeting. In this case, phage display can be extremely useful in identifying novel peptides with higher selectivity. The combination of several peptide ligands or peptide with other types of ligands should be expected to be created in the lab, with the huge potential to improve therapeutic efficacy in the clinic. Despite indisputable progress, the large number of variables such as lack of homogeneity and complexity of the biological environment, and our relatively limited understanding of their regulating elements, various process parameters, such as energy supply for the production of micro-emulsion, vessels size, impeller speed, temperature, agitation time, sonication time affect the nanomedicine production at industrial level, have put off the clinical implementation of theranostics systems.

4.6 | Growth factor as a target

The ability of growth factors to target the cancer cell-expressed receptor has been successfully utilized. Cancerous cells overexpress

numerous growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), and basic fibroblast growth factor (FGF-2), which specifically interacts with antibody/ErbB-2/HER2. Among these growth factors, EGFR has a great affinity for anti-EGFR; researchers are more interested in selecting it as a drug delivery and diagnosis marker. Figure 5b shows the schematic illustration of anti-EGFR Fab conjugated liposomal nanoparticles synthesis. One of the structural components of the liposome is DSPE-PEG-maleimide, which is involved in making bound with the thiol group of reduced Fab′ fragment with high efficacy. The binding efficacy in terms of IC50 (M) of Fab′ fragment and liposome- Fab′ fragment with EGFR target (sEGFR501.Fc.) was found to be 5.6×10^{-9}, 23×10^{-9}, respectively (Zhai et al., 2015). Because of the covalent linkage of the thiol-maleimide process, the conjugation remained stable for at least 2 months at 4°C. The EGFR is a high molecular weight compound with an extracellular N-terminal antibody-binding domain, a hydrophobic transmembrane region, and an intracellular C-terminal tyrosine kinase domain. The antibody-binding part effectively binds with EGFR, resulting in dimerization and cellular internalization of the receptor via the tyrosine kinase signaling pathway or clathrin-mediated pathway. Several growth factor-based nanomedicines have been approved for preclinical trials based on promising research results in vitro and in vivo systems. (Gridelli et al., 2010). Researchers are interested in developing a nanocarrier using an epidermal growth factor as a native ligand for targeting EGFR-expressing cancer cells. Anticancer drugs such as carmustine, gemcitabine, doxorubicin, and paclitaxel have been widely transported using anti-EGFR linkers and lipid as carriers in various model systems (Kuo & Liang, 2011). The EGF is a short peptide chain and is made up of 54 amino acid residues with a molecular weight of 6 kDa. It is a small molecule compared to an antibody which enhances the choice as an anchoring molecule for the development of the nanosystem. Ahlgren et al. explored the potential of EGF coupled with PEG-stabilized lipid nanodisks for anticancer drug curcumin delivery. The lipodisks were prepared using a dissolution of DPPC, cholesterol, NHS-PEG_{3400} (2:2:1) in the organic phase, dried, and subsequently hydrated with an aqueous solution at 60°C for 1 h. After that, EGF was conjugated to the lipodisks surface by replacing NHS-PEG_{3400}-DSPE with EGF-NHS-EGF_{3400}-DSPE in an aqueous solution (Figure 5c). In vitro study revealed that EGF-coupled lipodisks effectively bound with EGFR expressing A-431 cells and facilitated the 1.5-fold accumulation of drugs compared to uncoupled lipodisks (Ahlgren et al., 2017). Lung cancer cells overexpress the EGF tyrosine kinases receptor, facilitating the inactivation of the MAPK/PI3K/Akt/STAT pathway, resulting in angiogenesis, metastasis, and resistance against chemotherapy. Majumder and Minko synthesized NLCs comprising a multi-component system, which includes EFG-TK inhibitor, gefitinib, luteinizing hormone release hormone (LHRH, cancer cell detection moiety), paclitaxel (anticancer drug), rhodamine (imaging agent), and siRNA (targeted to mRNA encoding EGF receptor). Trilaurin (solid lipid), α-tocopherol (liquid lipid), DSPC (Emulsifier), DSPE-PEG-2000 (surfactant), and DOTAP (cationic lipid) are the structural components of the NLCs. The DSPE-PEG-LHRH peptide was synthesized using catalyst triethyl amine and was added in the lipid solution while synthesis of the carrier. However, anionic SiRNA was coupled with cationic lipid -DOTAP whose linker was exposed on the surface of the carrier. The results demonstrated that this multi-component delivery system has much higher anticancer efficacy (5–10-fold) than individual components applied separately, which depicts the strong detecting ability of the system (Majumder & Minko, 2021). Overall, anti-EGFR Fab′ and EGF conjugated NLBCs can bind to human tumor cells receptor with high specificity and selectivity. Importantly, the binding causes the nanocarrier to be internalized through the receptor. Further, the binding of the anti-EGFR Fab′ fragment on the surface of the lipid carrier did not significantly affect the size of the final formulations. It is also noticed that when antibody has been conjugated directly to the surface of PEG-NLBCs, the binding affinity with the targeted molecule decreases significantly. The fact that explains the above phenomenon is a steric hindrance produced by PEGylation which reduces the binding affinity of the conjugated molecules. Further, certain disadvantages are associated with anti-EGFR, such as expensive, poor pharmacokinetics, and tissue permeability and needing considerable effort to produce them. Moreover, like peptides, and antibodies used as targeting agents, EGF is also sensitive to harsh conditions. As a result, organic solvent exposure and ultrasound sonication, freeze-thawing must be avoided while preparing these conjugates.

4.7 | Galactose

Malignant cells overexpressing numerous receptors on their cell surface have a high affinity for protein molecules, and these receptors are commonly known as lectin receptors (Bies et al., 2004). Lectin-mediated drug delivery uses endogenous ligands such as lactose, fructose, fucose, mannose, and galactose (Kesharwani et al., 2011). Among them, galactose is one of the most utilized ligands targeting lectin molecules. Its free hydroxyl group at the C1 position interacts with lectin molecules with high affinity. Wang et al. successfully utilized the asialoglycoprotein receptor present on the hepatocytes that can especially recognize terminal β-D-galactose residue. They utilized liposomal-based nanocarrier using modified lipid components to efficiently detect and deliver doxorubicin to asialoglycoprotein overexpressed hepatocytes. The structural components, including doxorubicin, liposomes modified with a novel galactosylated lipid (CHS-ED-LA), cleavable PEG-lipid (PEG2000-CHEMS), HSPC, were used to synthesize different liposome nanocarriers. Namely, conventional liposome (CL), galactosylated liposome (GaIL), pegylated liposome (PEG-CL), pegylated galactosylated liposome (PEG- GaIL) are exploited to encapsulate the doxorubicin. The tumor weight in gram (g) of H22 tumor treated cells with various formulations, doxorubicin solution, CL-DOX, GaIL DOX, PEG-CL DOX, PEG-GaIL DOX were compared and found to be 0.362 ± 0.127, 0.198 ± 0.112, 0.221 ± 0.129, 0.114 ± 0.048, and

0.062 ± 0.033 g, respectively. PEG-GalL DOX significantly reduced tumor weight, suggesting the formulation effectively detects malignant cells. Jain et al. constructed galactose-decorated SLNs to improve target competency associated with an anticancer drug with a similar approach. The presence of lectin molecules on the surface of adenocarcinoma human alveolar basal epithelial cells enabled the galactose conjugated SLN to interact precisely. It also enhanced the bioavailability of the drug. The chemistry between solid lipids (Soya lecithin) containing stearyl amine and galactose molecule involved galactose ring-opening in the presence of acetate buffer at pH 4.0, at 60°C. Subsequently, galactose aldehyde was conjugated with an amino group of the stearyl amine through EDC–NHS chemistry, resulting in an amide bond formation between them (Figure 5d). The results of the cell cytotoxicity assay were backed up by quantitative cell uptake studies, which demonstrated a 1.5-fold increase in drug uptake when galactose conjugated SLNs were incubated with A549 cells; it had a greater absorption of drugs than SLNs and free drugs. The enhanced bioavailability of the anticancer drugs revealed the biomarker-based detection with selectivity through galactose conjugated SLNs (Jain et al., 2015). The above studies revealed that the drugs incorporated into galactosylated NLBCs, compared to NLBCs alone, possess increased bioavailability, stability, solubility, and encapsulation efficiency with no side effects of the anticancer drug in the biological system. However, the addition of galactose on the surface of NLBCs decreases the zeta potential and percent encapsulation efficiency. At the same time, the size of the final nano-bioconjugate increases, suggesting the system's stability could be affected. Percentage encapsulation efficiency reduction of final nano bioconjugate might be due to the loss of the surface adsorbed functionality in the acetate buffer.

4.8 | Monoclonal antibody

The major limitations of free monoclonal antibodies include enzymatic degradation, organ toxicity, poor solubility, and reduced bioavailability. Using a carrier system, such as polymeric or lipid-based nanocarriers, is a common way to overcome the above limitations. The use of nanocarrier also reduces the drug doses required to achieve the same therapeutic efficacy. The avidin–biotin interaction, one of nature's strongest noncovalent interactions, has been used as a carrier for delivering several pharmacological agents such as small molecules, monoclonal antibodies, vaccines, proteins, and nucleic acids. Avidin is a tetrameric glycoprotein that consists of four repeating units that bind to biotin with high selectivity and affinity (K_d 10^{-15}M). Biotin receptors overexpress many cancer cells, making them a potential tumor-targeting moiety. For instance, Lv et al. used biotin-functionalized poly (ethylene glycol)-b-poly (-caprolactone) nanocarrier carrying the chemotherapeutic agent doxorubicin (DOX) and the chemosensitizer quercetin (QUT) (BNDQ) to deliver two medicines to tumor cells at the same time. The incorporation of biotin on the nanocarrier's surface did not affect the size, charge, and polydispersity index, proving the stability of the final nano bioconjugate. The IC50 value of inducer BNDQ in MCF-7 cells was 0.05, 0.12 times lower than DOX (DOX + QUT), respectively. Also, compared to non-biotin conjugated nanocarrier, BNDQ has been shown to boost in vivo anticancer treatment efficacy (>3-fold).(Lv et al., 2016). However, avidin binds nonspecifically to other molecules because it has a positive charge at physiological pH. As a result, other types of avidin, such as streptavidin and neutravidin, are preferred. Streptavidin, unlike avidin, is not a glycoprotein and has a lower pI (Isoelectric point), which precludes it from interacting with sugar receptors. Currently, most of the research based on nanomedicines, antibody fragments, or antibodies is being highly utilized for cancer theranostics. The US Food and Drug Administration (FDA) has also approved antibody-based immunotherapies such as cetuximab and trastuzumab. The ligands (antibody or fragment of antibody) have been decorated on NLBCs or directly conjugated to bioactive molecules for diverse applications, including imaging, detection, and drug delivery in a controlled and precise manner. Warlick et al. employed biotin/neutravidin aptamers to efficiently attach to the NHS-PEG3400-Mal monomer of the nanoparticle through thiol conjugation and internalize anti-HER2-modified NPs in HER2-overexpressing cells. In this experiment, trastuzumab was biotinylated and attached to the neutravidin linker. The findings suggest that nanoparticles coupled with an antibody could be useful as a selective drug delivery system for a tumor that expresses a specific antigen (Wartlick et al., 2004). Furthermore, various binding techniques are now possible because of advancements in genetic engineering. For instance, the combination of streptavidin (monoclonal antibody), a single-chain variable fragment composed of the recombinant fusion protein (FP), can specifically bind with the same cellular epitope and drug-loaded nanoparticles, which is collectively known as a pretargeting strategy. Parker et al. investigated the binding of streptavidin-scFv FP by biomarker, tumor-associated glycoprotein 72 (TAG-72) present on leukemia cells, followed by pretargeting biotinylated nanoparticles. The Jurkat (T-leukemia) cells were treated with an FP, consisting of four anti-TAG-72 scFv tagged streptavidin and biotin-functionalized polystyrene nanoparticles (biotin-PEG-polystyrene nanoparticles). Results suggested that incorporating monoclonal antibodies on the PEG-based nanoparticles enhanced the twofold internalization efficiency in the leukemic cell line. However, multiple interactions between FP and nanoparticles result in aggregate formation on the cell surface, preventing the endocytosis of leukemic cells. Hence multivalent interactions between biotin-functionalized polystyrene nanoparticles and streptavidin-scFv FP provide an additional advantage (Parker et al., 2017). Similarly, Notabi et al. have chosen Cetuximab (anti-EGFR) as the targeting antibody that interacts with human EGFR with great affinity, suppressing cancer cell proliferation induced by this receptor. The EGFR gene is overexpressed in various solid tumors, making it a potential target for tumor-specific drug delivery for cancer therapy. The lipid NP surface was conjugated to both an albumin stealth corona and anti-EGFR targeting antibodies through thiol conjugation utilizing a one-step synthesis process. Albumin coating provides stability to lipid nanoparticles in serum and reduces

nonspecific cell interaction. Furthermore, antibody conjugated stealth lipid NPs reduced the nonspecific binding 1000-fold compared to free drugs. and their cargo to be retargeted by the cells (Notabi et al., 2021). The development of monoclonal antibodies has revolutionized the pharmaceutical technology in combating various disease therapies, including cancer therapy.

4.9 | Immunotherapy

Cancer immunotherapy, a potential and commonly used method of monotherapy, uses immune stimulants and modulators to treat cancer-related immune dysregulation in a specific manner. The targeted drug delivery system (DDS) can actively administer drugs by linking NP's surface with effector molecules, permitting DDS to reach and penetrate cells overexpressing a specific receptor, then releasing enclosed drugs in a sustained and controlled manner in the target cells. Antibodies, peptides, and affibodies are some examples of potential effector molecules. Lipid-based nanocarriers are currently being exploited as immunotherapeutic vehicles and can transport material and induce immune responses. Hence, functionalizing lipid-based nanocarriers with immuno-active molecules plays a pivotal role in cancer diagnosis and therapy. Immunostimulatory agents such as anti-CD137 and an IL-2 stimulate strong anticancer activity by activating the cytokines production, granzyme, and cytotoxic lymphocytes, but at the same time, they induce systematic toxicity. Zhang et al. developed the techniques in which the fusion of anti-CD137 and an IL-2-Fc was conjugated to the liposomal surface to overcome the cytotoxic effect of the immune modulators. They synthesized the liposome using DSPE-PEG, DOPC, and DSPE-PEG-maleimide cholesterol as structural components. The fusion of anti-CD137 and an IL-2-Fc was coupled with DSPE-PEG-maleimide through a thiol reaction. The liposomal conjugated anti-CD137 and an IL-2-Fc fusion were found to have a 4.5-fold increase in absorption when total anti-CD137and IL-2-Fc signal was quantified in the tumor environment compared to free anti-CD137 and an IL-2-Fc with reduced systematic toxicity (Y. Zhang, Li, et al., 2018). Antibodies against programmed death-ligand 1 (PD-L1) have been demonstrated to be useful in solid tumors. A cell surface receptor known as PD-1 plays a critical role in immune system downregulation by decreasing T-cell inflammatory activity. Xu et al., in their study, demonstrated the effectiveness of an antibody conjugation approach in which PEG-PCL NPs were conjugated with a PD-L1 mAb before being loaded with docetaxel (DOC). The MTT assay was used to assess the cytotoxic activity of NPs on MKN45, MGC803, and HGC27 cells. The PD-L1 mAb -conjugated DOC-PEG-PCL NPs could be used for targeted immunotherapy. The DOC-PEG-PCL-IgG NPs' loading capacity (LC) and encapsulation efficiency (EE) were 46.6% and 58.6%, respectively. In contrast, the DOC-PEG-PCL-mAb NP's EE and LC were only 48.8% and 46.5%, respectively. Though DOC-PEG-PCL-mAb NPs have less encapsulation efficiency than DOC-PEG-PCL-IgG NPs', it has higher cancerous cell (HGC27) apoptosis. DOC-PEG-PCL-mAb NPs and DOC-PEG-PCL-IgG NPS have cell apoptosis fractions of 37.4 ± 0.23% and 17.2 ± 0.13%, respectively (Xu et al., 2018). Lee et al. have devised a new technique for breast cancer treatment that uses "click chemistry" and aided immune cell targeting to release doxorubicin (DOX)-loaded nanoparticles to regions of the tumor that are inadequately vascularized. Immune cell-targeting antibodies CD11b were functionalized with trans-cyclooctene (TCO) by a TCO-PEG4-NHS linker, and 1,2,4,5-tetrazine (Tz) was coated on DOX-laden mesoporous silica nanoparticles (DOX-MSN) to facilitate conjugation via the media reaction. MSN was first prepared, and the extra cationic surfactant (CTAB) serving as a porogen was removed with ammonium nitrate. Then, (3-aminopropyl) triethoxysilane (APTES) was used to modify MSN with amine groups. Further, the orthotopic 4T1 tumor mouse model was treated with TCO-modified anti-CD11b and DOX-MSN-Tz in a sequential manner. These successive infusions were shown to minimize rapid removal from circulation and induce a large proportion of DOX-MSN-Tz into the tumor when compared to pre-conjugates anti-CD11b-TCO and DOXMSN-Tz. As a result of this conjugation method, deep tumor penetration and homogeneous distribution of lipid nanocarriers result in increased DOX therapeutic efficiency (Lee et al., 2019). However, whole antibodies sometimes cause cytotoxicity. Hence, antibody fragments can be used to substitute for targeted drug delivery. Nikkhoi et al. explored the possibility of using a HER2-antibody fragment to treat breast cancer. He utilized the VHH-domain, bivalent and bispecific, and coupled it with liposomes. A thiol-maleimide reaction catalyzed with *N*-hydroxysuccinimide (NHS) was used to attach antibody fragments, VHH on PEGylated liposomes which is a common approach for antibody attachment. Two single-domain antibody fragments fused splicing by overhang PCR to produce bispecific VHHs. The gene encoding VHHs are amplified by two set of primer containing hinge sequence. VHHs are normally fused together by a 10 amino acid or larger linker so that both binding sites of the bivalent VHH bind the target at the same time. In this study, the llama's hinge sequence was used to connect the two VHH and these two VHHs bind to the HER2-positive cell lines in a specific way. Bivalent, biparatopic VHH-targeted liposomes showed a considerably higher affinity when compared to monoparatopic and monovalent VHH-targeted liposomes (Nikkhoi et al., 2018). The majority of currently available antibodies are monospecific, and they can only recognize, bind, and interfere with one antigen. However, some complex diseases, such as cancer, are multifactorial and require treatment platforms that are coupled and synergistic. In addition to their multifactorial nature, cancerous cells are highly mutagenic and acquire resistance to monospecific or monoparatopic monoclonal antibody therapy. Antibodies that combine more than one epitope increase antibody affinity by cross-linking antigens and minimize the chance of epitope extinction due to genetic drift. Further, antibody fragments are stable, small, and robust antigen binding molecules with high affinity than conventional antibodies and generally lack glycosylation, allowing their production in the expression systems, which provide savings in time and cost. Immunotherapy strengthens the body's natural defenses in the treatment of cancers. Antibodies and other targeted medicines are becoming increasingly

TABLE 2 List of NLBCs formulation for cancer diagnosis and therapy

S. no.	Types of NLBCs	Lipids	Therapeutic agent	Diagnostic/imaging agent	Cell line/animal	References
1	Liposome	DPPC, D-alpha-tocopheryl polyethylene glycol 1000 succinate	Docetaxel	QDs	Charles Foster rat	Sonali et al. (2016)
2	Liposome	DOPE-Rhodamine, DOPC, DSPE-PEG-2000, DSPE-PEG (2000) folate	NR	Gadolinium (III)-DOTA-DSA	Balb/c mice	Jung et al. (2012)
3	Liposome	BAT-PEG-DSPE, HSPC, cholesterol, DSPE-PEG-2000	NR	^{64}Cu/PET imaging	Male FVB mice	Seo et al. (2008)
4	Liposome	DSPE), DOTA-NHS ester	NR	^{64}Cu/PET imaging ^{52}Mn/PET imaging	CT26 tumor-bearing mouse	Jensen et al. (2018)
5	liposome	DEPC, DSPC, DSPC-PEG2000	Doxorubicin	BR-55 (Ultrasound contrast agent)	Orthotopic prostate tumor animal model	Helbert (2021)
6	Liposome	DSPC, DSPE-PEG-2000, and cholesterol	Methyl aminolevulinat	Methyl aminolevulinat	SKOV3 cell line	Luan et al. (2021)
7	Liposome	DPPC, PEG2000-DSPE), DSPE-PEG-2000-folate	Paclitaxel and vinorelbine	Folic acid/99 mTc (SPECT)imaging	H1299 cells	Karpuz et al. (2020)
8	Liposome	DPPC, DSPE-PEG, cholesterol, lecithin, DPPE-PEG-FITC	Cisplatin & Gemcitabine	Folic acid, Fe_3o_4-coated dextran (MRI contrast agent)	Folic acid, Fe_3o_4-coated dextran (MRI contrast agent)	Pakdaman Goli et al. (2021)
9	NLCs	Cetyl palmitate, coconut oil, myverol	Camptothecin	QDs	Melanome cell/Female nude mice	Hsu et al. (2013)
10	NLCs	Glyceryl monostearate, oleic acid	Paclitaxel	Stearyl-2-amino-2-deoxyglucose (2-DG), fluorescein/Cypate	MCF-7 tumor bearing mice	J. Chen et al. (2012)
11	NLCs	Glyceryl monostreate, cetyl palmitate, Stearic acid, oleic acid, Tween-80	Silymarin	Barium sulfate/X-ray imaging	Caco-2 cell	Chaudhary et al. (2015)
12	NLCs	Stearic acid/oleic acid	Paclitaxel	Folate/Technetium-99m tricarbonyl complex (99mTc(CO)3+).	MCF-7/human epithelial cervix adenocarcinoma cell line	Ucar et al. (2017)
13	NLCs	Glyceryl monostearate, oleic acid, and soya phosphatidylcholine	paclitaxel	QDs(CdTe/CdS/ZnS)	H22 tumor	Olerile et al. (2017)
14	SLNs	Pluronic® F-68, formamide, glyceryl trimyristate,	NR	Fe3O4 (MRI contrast agent	HT29 colon adenocarcinoma cell line	Muñoz de Escalona et al. (2016)
15	SLNs	Soy lecithin, Compritol® 888 ATO (glyceryl behenate), Tween 80	Curcumin	Technetium-99 m (99mTc)	New Zealand rabbit	Ayan (2017)
16	SLNs	Lecithin, stearic acid	NR	(^{64}Cu/PET)	Mouse	Andreozzi et al. (2011)

TABLE 2 (Continued)

S. no.	Types of NLBCs	Lipids	Therapeutic agent	Diagnostic/imaging agent	Cell line/animal	References
17	SLNs	Stearic acid, sodium taurocholate	NR	Superparamagnetic iron oxide (MRI)	Dawley rats	Peira et al. (2003)
18	SLNs	Triglycerides and cholesterol	Paclitaxel-siRNA combination	QDs	Human lung carcinoma cells	K. H. Bae et al. (2013)

Abbreviations: BAT, 6-[p-(bromoacetamido)benzyl]-1,4,8,11- tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; CHS-ED-LA, (5-cholesten-3β-yl) 4-oxo-4-[2-(lactobionyl amido) ethylamido] butanoate; DEPC, 1,2-dierucoyl-sn-glycero-3-phosphocholine, 1,2-distearoyl-sn-3 phosphoethanolamine-*N*-methoxy (polyethylene glycol)2000–methoxy(PEG-2000)-DSPE; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPE, dioleoylphosphatidylethanolamine; DOTA-1,4,7,10, tetraazacyclododecane-1,4,7,10-tetraacetic acid; DOTAP, 1,2-Dioleoyl-3-trimethylammonium propane; DPPC, dipalmitoyl phosphatidylcholine; DPPE, dipalmitoyl-phosphatidylethanolamine; DSA, 2-{4,7-bis-carboxymethyl-10-[(*N*,*N*-distearylamidomethyl-*N*′-amido-methyl]; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; DSPE, 1,2-distearoyl-sn-glycero-3-phosphoetanolamine; EDC, ethyl-3-(3-dimethylamine propyl) carbodiimide; EPOPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine; FITC, fluorescein isothiocyanate; HSPC, L-α-phosphatidylcholine hydrogenated (Soy); MSPC, monostearoyl phosphatidylcholine; NLC, nanostructured lipid carrier; NHS, *N*-hydroxy succinimide; PEG, polyethylene glycol; PEG-PE, polyethylene glycol-phosphatidyl ethanolamine; PEG2000-CHEMS, PEG-lipid methoxy polyethyleneglycol 2000-cholesteryl hemi succinate; QDs, quantum dots; SLN, solid lipid nanoparticle.

significant in cancer treatment. Many clinically effective antibodies have the ability to modulate tumor signaling. Antibodies also have immunomodulatory capabilities, which means that they can boost the development of antitumor immune responses by directly activating or inhibiting immune system components (Table 2).

4.10 | Diagnostic/imaging agent

In the case of various tumors, diagnosis using NLBCs is a potential strategy because the nanocarriers facilitate the transport of imaging agents at the targeted site. Rhodamine 123 is a fluorescent-based dye conjugated with various NLBCs and used to image liver tumors using MRI. Albumin-binding Gadolinium (III) complex is an MRI contrast agent for the xenograft model of breast cancer. Cyanine dyes, tiny fluorescent molecules that can absorb in the UV, visible, or near-infrared range, have been studied extensively as photodynamic therapy photosensitizers. indocyanine, a disulfonated heptamethine cyanine with absorbance at 780 nm, has been incorporated into nanostructure and used to diagnose cancer cells. The various radionuclides such as gamma emitters (99mTc, iodine-123 ^{123}I, indium-111 ^{111}In), positron-emitting radionuclides (gallium-68 [68 Ga], fluorine-18 [18 F]), and others, both of the positrons from radionuclides are used for PET (positron-emitting tomography) imaging and beta-particles such as copper-64 (64Cu), are labeled with various targeted molecules including aptamer for cancer imaging. The emitted signals from radioactive decay of radionuclides can be detected using a gamma-camera through single-photon emission tomography (SPECT) or hybrid SPECT/CT (computer tomography) and PET/CT system. Magnetic fluid-loaded liposomes (MFLs) were produced and targeted in animal models, that is, mice with rhodamine-labeled superparamagnetic hybrid liposomes. The main components of MFLs are pegylated phospholipid and superparamagnetic nanocrystals of maghemite (γ-Fe2O3) which forms unilamellar vesicle. The employment of a fluorescent marker, that is, rhodamine and effective magnetic resonance contrast agents and maghemite nanocrystals to detect MFL accumulation in solid human prostate cancer tumors (Martina et al., 2007). The integration of lipid nanocarrier (medium-chain triglycerides with PEGylated surfactant) with QDs, Forster resonance energy transfer (FRET), fluorescent dyes, and other fluorescents/imaging molecules that help in the detection of cancer. For the first time in the year 2006, the efficient FRET was inserted in the lipid nanocarrier for in vivo imaging. Two different dyes were synthesized, that is, lipophilic cyanine 5.5 and 7.5, and these dyes were encapsulated in NLBCs according to the required concentration for efficient FRET. This unique integrated nanocarrier helped quantitative in vivo imaging directly in blood circulation, liver, and tumor xenografts (Bouchaala et al., 2016). A list of NLBCs (liposome, SLNs, and NLCs) comprising both therapeutic and imaging agent are illustrated in Table 3. Despite several efforts, only a small percentage of nanomedicine is approved for commercialization. One of the major hurdles in

achieving a higher success rate in developing nanomedicines is the translational gap between animal and human species. This gap has emerged due to the physiological and pathological disparity between these species. Moreover, patients' heterogeneity can also hinder the efficacy of nanomedicines and is a subject of research on how they interact with the actual diseased population in real time. This knowledge could help design and formulate nanocarriers and propel the field forward.

5 | CLINICAL STUDIES

The transition of nanomedicines from preclinical findings to clinical research necessitates designed models and approaches. This further help to predict lipid fate inside the body, which boosts clinical applications of the nanocarrier system. The liposome-based delivery system is employed in clinical trials to transport bio-actives molecules such as medicine, genes, and other low molecular substances. Several characteristic features of NLBCs, such as high biocompatibility and biodegradability, offered huge potential in the transportation of numerous anticancer medicines and arose new hope for cancer patients. Grb-2, a protein overexpressed on the surface of leukemic cells, has been successfully targeted with an oligonucleotide-liposomes complex. Grb-oligodeoxynucleotide is used as an antisense oligodeoxynucleotide and specifically binds with the Grb2 sequence resulting in hampering protein expression and cell proliferation. After achieving fruitful results in vitro and in vivo, the oligonucleotide-based nanomedicine has entered the preclinical trials and completed Phase 1 trials. Recently, nanomedicine under the brand name BP1001 combined with ventoclax plus decitabine and BP1001 plus decitabine has been recruited for Phase 2 clinical trials (https://clinicaltrials.gov/). MM-302 is a pegylated liposome loaded with doxorubicin that precisely targets the cells and reduces the human body's side effects. Espelin et al. combined the use of trastuzumab along with MM-302 for efficient targeting while excluding the adverse side effect in the HER2-overexpressed breast cancer cell line. MM-302 and trastuzumab have different specificity for HER-2 receptors and therefore bind with a different domain and simultaneously enhance the therapeutic efficacy in receptor overexpressed tumor cells. It has also been observed that trastuzumab does not interrupt the action mechanism of doxorubicin, resulting in DNA damage and cell cycle arrest. Similarly, MM 302 does not disrupt the working mechanism of trastuzumab that blocks the P-Akt signaling pathway. In HER2-overexpressed xenograft models, MM-302 and trastuzumab have exhibited synergistic antitumor effects (Espelin et al., 2016). On the other hand, clinical trials of the formulation mentioned above found no improvement over control according to DMC (Data Monitoring Committee) and confirmed via futility analysis. Several ligand-decorated nanomedicines are undergoing clinical trials at various phases, and some of them are listed in Table 3.

TABLE 3 List of ligands-decorated nanomedicines in different phases of clinical trials

S. no.	Liposomal nanocarrier system	Therapeutic molecules	Targeting ligands	Cancer type	Clinical status	Reference/sources
1.	Anti-EGFR immunoliposomes	Doxorubicin	Cetuximab Fab Fragment	Breast cancer	Phase II	clinicaltrials.gov/ct2/show/NCT01702129
2.	Liposomal doxorubicin	Doxorubicin	Anti-CD19	B lymphoma	Preclinical Stage	Allen et al. (2005)
3.	RGD/Tf-liposome	Paclitaxel	RGD/Tf	Brain glioma	Preclinical Stage	Qin et al. (2014)
4.	αvβ3-integrin-liposome	NR	cRGD	4T1 murine mammary carcinoma	Preclinical Stage	Sofias et al. (2020)
5.	Liposomal Oxaliplatin/Folinic Acid/5-Fluorouracil	Oxaliplatin	Transferrin ligand	Gastroesophageal Adenocarcinoma	Phase II	https://clinicaltrials.gov/ct2/show/NCT00964080?term=NCT00964080&draw=2&rank=1
6.	Atu027	siRNA	Protein kinase N3	Solid tumor	Completed	https://clinicaltrials.gov/ct2/show/NCT01808638?term=NCT01808638&draw=2&rank=1
7.	SGT53-01	p53 gene	Antibody fragment	Solid tumor	Completed	https://clinicaltrials.gov/ct2/show/NCT00470613?term=NCT00470613&draw=2&rank=1

6 | CONCLUSION

More than 200 different types of cancers affect the human population socially, emotionally, and financially worldwide. Numerous natural and chemically synthesized anticancer medicines have been screened, but solubility and toxicity are the major concerns. These constraints can be conquered using various ligands–nanocarrier conjugates that are well explored in this review. Also, this review has elaborated that perforated vascular systems facilitate the passive targeting of nanocarriers. In recent decades, many types of surface receptors overexpressed on the surface of tumor cells have been discovered. Their interactions with target ligands have been demonstrated, leading to the active targeting of nanocarriers. Also, various ligands and lipid nanocarriers have been well discussed and have shown promising results in drug delivery and detection of target cells without causing harm to healthy cells. Due to recent scientific endeavors, several nanocarriers with ligands are undergoing preclinical or clinical studies. Some have even progressed in different phases of clinical trials, and a few of these nanomedicines will be ready for human use shortly. Various cancer stem cells overexpressed certain biomarkers, including CD133, epidermal cell adhesion molecule (EpCAM), and aldehyde dehydrogenase 1 (ALDH1). In the case of hepatocarcinoma, colorectal cancer, and gastric cancer, a membrane-bound protein called Eph B2 (receptor tyrosine subfamily) is expressed significantly. Further various interleukins comprising IL-4Rα, IL-1β, and IL-8 are membrane-bound and secretory proteins produced at high levels in various carcinoma. Therefore, designing and developing ligands against these biomarkers would be highly interesting. Another approach can be antibody engineering, especially monoclonal antibody production, by inducing whole protein, fragment, or sequence of a specific domain of a biomarker of interest. These approaches may boost the targeting and diagnosis affinity. Metal-organic frameworks (MOFs) are materials with adjustable porosities, functional surfaces, and bulk conjugated backbones of metal ions and organic linkers. MOFs have been reported to have exchange abilities between the extra framework ions and various external guest species. These MOFs delivered via lipid nanocarriers can also sense and diagnose colon cancer, usually showing sevenfold higher copper concentration than normal cells. These approaches may boost the formulations' targeting and diagnosis ability against various carcinoma. We further conclude that various ligands need to be engineered, which may pave the way toward detecting and targeting malignant cells.

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DATA AVAILABILITY STATEMENT

Research data are not shared.

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