Targeted Liposomal

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The targeted liposomes have been developed and utilized to deliver drugs to the tumor or tumor microenvironment with minimal non-specific distribution in normal tissues or organs. Various tumor-targeting ligands, such as small molecules, oligonucleotides, peptides, monoclonal antibodies (mAbs) and antigen-binding fragments (Fabs), have been conjugated with liposomes.

Keywords: combined chemotherapies ; targeted liposomes ; triple-negative breast cancer

1. Introduction

Triple-negative breast cancers (TNBCs) are the breast cancers that lack expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER)-2/neu. Currently chemotherapeutic agents are the most common clinical treatment strategies employed to suppress tumor growth, but TNBC patient responses differ from case to case. For instance, drug resistance due to drug efflux [1][2][3], apoptosis dysregulation [4][5], activation of survival, growth and invasion signaling pathways [6] or others [7][8] significantly limits their clinical efficacy and also leads to tumor recurrence and progression [9]. In addition, patients usually suffer from side effects, such as fatigue, emesis, hair loss, and anemia, due to a lack of an effective tumor targeting method.

The U.S. Food and Drug Administration (FDA) has approved liposomes as a drug delivery vehicle with guidance of “Chemistry, manufacturing, and controls; human pharmacokinetics and bioavailability; and labeling documentation” (FDA-2016-D-2817). The targeted liposomes have been developed and utilized to deliver drugs to the tumor or tumor microenvironment with minimal non-specific distribution in normal tissues or organs. Various tumor-targeting ligands, such as small molecules, oligonucleotides, peptides, monoclonal antibodies (mAbs) and antigen-binding fragments (Fabs), have been conjugated with liposomes. For example, the anti-epidermal growth factor receptor (EGFR) [10][11], HER2 [12] and vascular endothelial growth factor (VEGF) [13][14] antibodies or peptides have been linked to liposomal system to deliver doxorubicin or other medicines to breast cancers and other tumors. The fibronectin-mimetic peptide-PR_b [15], estrogen receptor-antagonist Tamoxifen [16] and peptide SP90 [17] have been used as linkers in liposomal drug formulation to treat breast cancers. Moreover, GAH mAb conjugated immunoliposomes have been fabricated to targeting deliver doxorubicin to treat human gastrointestinal cancers [18].

The EGFR, which stimulates the cancer proliferation via PI3K/RAS signaling, the repair of DNA damage and metastasis [19][20][21][22][23], is overexpressed in various tumors, e.g., TNBC (52–54%) [24][25], lung cancer (40%) [26][27], glioblastoma (50%), head and neck cancer (80–90%) [24][25][28], ovarian, cervical, bladder, gastric, endometrial and colorectal cancers [29]. EGFR is more predominant in TNBCs than other breast cancers [24][30], and usually correlates with tumor invasion and poor prognosis. From this perspective, anti-EGFR mAb was utilized in this study as a ligand to target TNBC. The targeted liposomal drug formulation is expected to prolong the circulation half-life and enhance the maximum tolerated dose.

Many innovative anti-cancer drugs failed in phase II clinical trials [31] although the pre-clinical results are promising. This could be attributed to the limitation of preclinical animal models such as lacking heterogeneity and tumor microenvironment. This challenge can be partially solved by applying patient-derived xenograft (PDX) models in the in vivo evaluation of the anti-tumor efficacy of new medicines. PDX models have been established by transplanting the cancerous cells or tissues from primary patient tumors and served as a good preclinical platform to predict the possible patient responses to new cancer medicine.

2. Targeted Liposomal Chemotherapies to Treat Triple-Negative Breast Cancer

Chemotherapies are still the major strategy to treat TNBC in clinics. We identified a new formulation of combined chemotherapies and also established a targeted delivery method for TNBC treatment, which could address the challenges

of drug resistance or poor clinical efficacy as well as treatment related toxicities. We have evaluated a highly potent drug and several standard chemotherapies for cancer treatment, including DM1, GC, AC, and PTX [32], and two combinations of these drugs. Combining standard GC and potent DM1 can kill over 90% TNBC cells with significantly reduced IC_{50} value and also effectively inhibit TNBC tumor growth in both cell line-derived xenograft models and patient-derived xenograft models. In addition to the improved cytotoxicity, GC and DM1 have different anti-cancer mechanisms so the combination could reduce the possibility of drug resistance development during long-term treatment compared to monotherapy. Therefore, the combination of GC and DM1 has great potential to treat TNBC.

We established and optimized the procedures of neutral liposomes synthesis to pack chemotherapies, surface tagging of TNBC-targeting antibody (mAb-Lipo), PEGylation, drugs packing, purification and characterization following the published guideline and protocols [33][34][35][36][37][38][39][40][41][42][43][44] with optimization. Non-targeting liposomes [45][46][47][48][49] have been used to deliver chemotherapies and other therapies, but the mAb-Lipo has multiple advantages, such as cancer-specific targeting, high packing capability with the developed all-in-one synthesis procedure, and high plasma stability and prolonged half-life with integrated PEG. Importantly, our surface tagging technology enables conjugating single or two (even multiple) antibodies to achieve dual-targeting to cover more patients with heterogeneous tumors. In addition to chemotherapies, the cationic liposomes encapsulated plasmid DNA (named as lipoplexes) have been evaluated in clinical trials for cystic fibrosis [50], non-small-cell lung cancer [51], metastatic melanoma [52][53], and epithelial ovarian, fallopian tube or primary peritoneal cancers [54] treatment.

Literature [25][55][56][57], clinical data [24][25][55] and our immunohistochemistry staining of patient tissue microarray show that EGFR is an excellent surface receptor in human [58][59][60] and mouse [61][62][63] TNBCs. For example, the anti-EGFR cetuximab and panitumumab are used in clinic to treat head and neck cancer [64][65][66] and colorectal cancer [67][68][69]. Moreover, the cetuximab mediates antibody-dependent cell cytotoxicity (ADCC) in the intratumoral space and primes adaptive and innate cellular immunity [70]. By tagging anti-EGFR mAb (cetuximab) to the surface of liposomes, we not only achieve TNBC tumor targeting but also could integrate the immunotherapy of the mAb. Of course, further investigation is needed to delineate the possible integrated anti-TNBC mechanisms of the tagged mAb and delivered GC and DM1 in future.

The TNBC xenograft models derived from various cell lines have been widely used in vivo to evaluate the tumor treatment efficacy. The PDX models are more advanced to evaluate new therapies as they have multiple advantages such as capturing TNBC heterogeneity and tumor microenvironment. For instance, PDX tumors can accurately recapitulate the phenocopy and mutation status of patient tumors, and resemble and maintain the biological behavior correlating with high metastasis, high heterogeneity and poor survival of TNBC patient tumors. Limited by the fresh patient tissues assessment and pathology analysis, many research labs have difficulty to establish in-house TNBC PDX models. We evaluated the Jackson lab commercial PDX lines and established a robust procedure to passage and maintain PDX lines in the research lab. The identified EGFR overexpressing PDX lines can be used as a good model to evaluate the therapeutic efficiency of newly developed therapies.

3. Conclusions

The combination of chemotherapies with different anti-cancer mechanisms (gemcitabine and mertansine in this study) has great potential to treat the highly aggressive TNBC. The technical challenges to apply combined chemotherapies, including circulation stability and side effects, can be overcome by the application of a targeted liposomal delivery vehicle. Importantly, different drug combinations can be easily adapted to this system for the treatment of recurrent cancer. Despite the promising results, the developed new formulation needs further evaluation in the future, such as pharmacokinetics, dosage optimization, metastatic tumor treatment and immune modulatory response.

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