Synthesis of Amphiphilic Block Copolymers to Deliver HSP90 Inhibitors for Colorectal Tumors

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ABSTRACT

Cancer therapy has attracted people attention for many years, especially for colorectal cancer. We have used Cetuximab (epidermal growth factor receptor inhibitor) to treat metastatic colorectal cancer, but it is no use in colorectal tumors harboring KRAS mutations. Recently, the research found that Heat shock protein 90 (HSP90) inhibitors have potential targeting KRAS mutant tumors. HSP90 is a molecular chaperone protein critical for tumor survive and growth. Here, we used amphiphilic block copolymers poly(ε-caprolactone)-graft-poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA-PCL) synthesized by ring-opening polymerization of ε-caprolactone. For drug delivery advanced application, these amphiphilic block copolymers had pH-sensitive characterization to release drugs at tumor site. In vitro cell viability showed HSP90 inhibitors reduced obvious cell growth. These results indicated that the use of the PDMAEMA-PCL loaded HSP90 inhibitors may be a potential method in cancer therapy.

Keywords: cancer therapy, colorectal cancer, drug delivery, nanoparticles, self-assembly

Different types of traditional nano-sized carriers, such as polymeric micelles, magnetic nanoparticles, nanotubes and dendrimers, etc., are being developed for various drug-delivery applications[1]. Nanoparticles drug delivery systems have attracted great attention as efficient tumor-targeting anticancer agents because enhanced permeability and retention (EPR) effect is the basis for the selective targeting of drugs to tumor [2]. Cetuximab (Erbitux) is used to treat colorectal cancer patients, but a main limitation of therapies that KRAS mutations are considered no response to Cetuximab [3]. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is an analog chemically derived Geldanamycin (GA) which is which acts as an anti-tumor agent. Recently, 17-AAG has been reported to target KRAS mutant tumors [4]. We demonstrated good small size and pH-sensitive amphiphilic block copolymers poly (ε-caprolactone)-graft-poly (2-(dimethylamino) ethyl methacrylate) (PDMAEMA-PCL) to deliver anticancer drugs.

The simple chemical synthesis route of PDMAEMA-PCL is shown in fig 1. The thermo-sensitive properties of PDMAEMA-PCL nanoparticles were studied in the range of 25–60℃. As shown in Fig. 2(a). On the other hand, the size of PDMAEMA-PCL nanoparticles was increased when decreasing pH of solution. As shown in Fig. 2(b).

A co-solvent evaporation method was used for the self-assembly of PDMAEMA-PCL block copolymers and drug encapsulation (Fig 3).

Fig 4. Illustrates that different D/P ratio had formed different size, encapsulation efficiency and zeta potential etc. We would like to choose the smaller size and better PDI value to treat tumor cells. Determination of EGFR expression in three human colorectal cell lines HT29, HCT116 and SW620 were measured by flow cytometry (Fig 5). We characterized SW620 (KRAS mutant) and HT29 (KRAS wild-type) untreated and treated with cetuximab or 17-AAG alone and in combination with chemotherapy drug 7-ethyl-10-hydroxycamptothecin (SN-38). 17-AAG could inhibit KRAS wild-type or mutant cells growth and proliferation compared to EGFR inhibitors cetuximab. A synergistic effect was observed for SN-38 in combination with cetuximab and 17-AAG (Fig 6).

In this article, we present pH-sensitive and small size nanoparticles as carrier to encapsulate 17-AAG. We anticipate that this platform will provide a new strategy for cancer therapy.

REFERENCES

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TABLES AND ILLUSTRATIONS

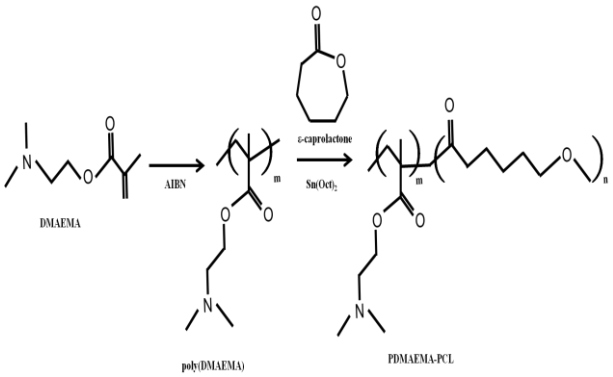

Figure 1. Chemical synthesis route of PDMAEMA-PCL. These amphiphilic block copolymers were synthesized by ring-opening polymerization of ε-caprolactone in the presence of PDMAEMA, AIBN as initiator.

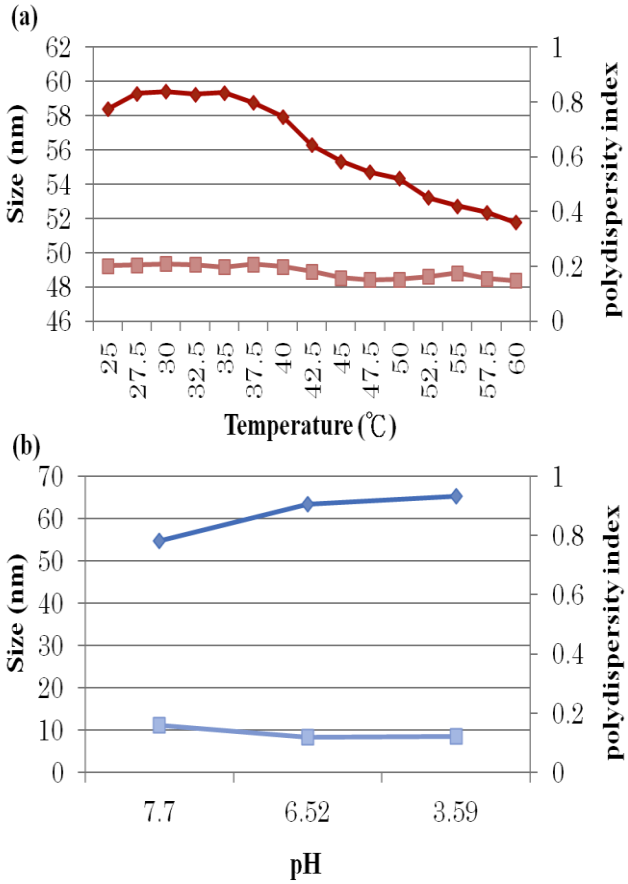

Figure 2. Evaluation of thermo- and pH-sensitive properties of PDMAEMA-PCL. (a) The experiment was studied in the range of 25–60℃, the size of PDMAEMA-PCL nanoparticles was slightly smaller when the temperature is increased. (b) The experiment was performed at 25℃. In acid pH environment, the amine groups of PDMAEMA moieties were protonated and resulted in the increase of nanoparticle size.

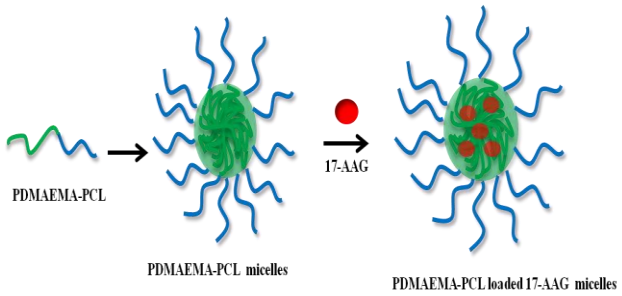

Figure 3. 17-AAG drugs were loaded into polymer micelles by co-solvent evaporation method. The solvent evaporation method consisted of the dissolution of the drug and polymer, and then this solution was added drop-wise to water. The mixture was then stirred at room temperature for 24 h and remainder of the organic solvent was removed by evaporation.

sample	D/P ratio	Mean size (nm)	PDI	E.E. (%)	Drug loading content (%)	Zeta potential(mV)
DMAEMA-PCL nanoparticle	---	58.39	0.203	---	---	55.1
17-AAG loaded nanoparticle	1/1	163	0.237	38.34%	12.78%	41
	1/5	118.2	0.291	59.43%	3.96%	32.3
	1/10	80.38	0.406	69.63%	2.32%	28.5

Figure 4. Characteristic of amphiphilic block copolymers used in this article. We chose low PDI value and high encapsulation efficiency as our material for later experiment. Encapsulation efficiency (EE %) was determined using below formula: (the total amount of drug in the nanoparticles / the total quantity of drug) ×100 %. Drug loading content **(%)** was calculated below formula: (the total amount of drug in the nanoparticles / the weight of polymer) ×100 %.

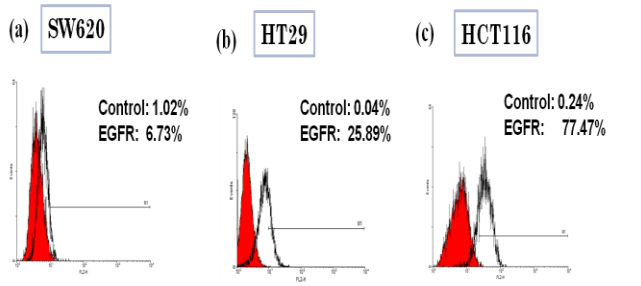

Figure 5. We chose three cell lines to measure EGFR expression by flow cytometry. Flow cytometry showed that HCT116 and HT29 expressed EGFR strongly whereas SW620 expressed EGFR weakly.

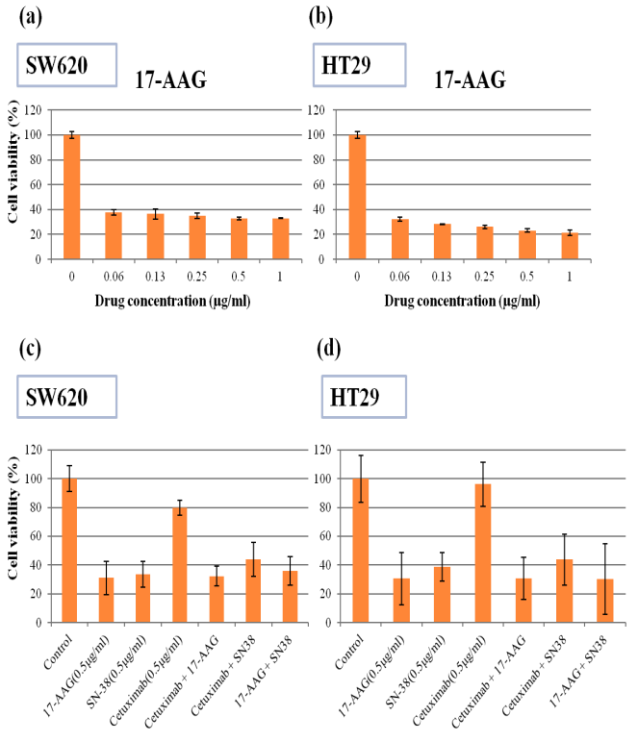

Figure 6. We chose two cell lines HT29 (KRAS wild-type) and SW620 (KRAS mutant). The top two panels (a) (b) denoted the inhibition rate of SW620 and HT29 cells viability in a dose-dependent manner. The bottom three panels (c) (d) showed a single drug or combined two different drugs cell viability. Cell viability slightly decreased when cell lines was treated Cetuximab only. We found that 17-AAG reduced cell viability similar with chemotherapy drug SN-38.