Journal of Colloid and Interface Science 451 (2015) 198–211

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

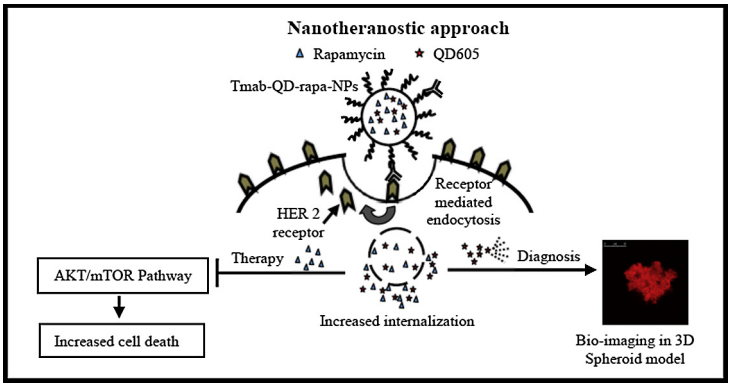
www.elsevier.com/locate/jcis

Trastuzumab guided nanotheranostics: A lipid based multifunctional nanoformulation for targeted drug delivery and imaging in breast cancer therapy

Priyambada Parhi, Sanjeeb Kumar Sahoo *

Institute of Life Sciences, Nalco Square, Chandrasekharpur, Bhubaneswar, India

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 January 2015
Accepted 27 March 2015
Available online 3 April 2015

Keywords:
Nanotheranostics
Rapamycin
Nanoparticles
Quantum dots
Trastuzumab

ABSTRACT

Nowadays, emerging aspects of cancer therapy involve both diagnostic and therapeutic modules in a single setting. Targeted theranostic nanoplatforms have emerged globally as frontier research for the improvement of cancer therapy. Trastuzumab (Tmab), a humanized monoclonal antibody is now being used to target human epidermal growth factor receptor-2 (HER 2) positive cancer cells. In the present study, we have analysed the imaging and theragnosis potentiality of Tmab functionalized lipid based nanoparticles (NPs) loaded with anticancer drug rapamycin and imaging agent (quantum dots) for targeted cancer therapy and imaging. The therapeutic evaluation of drug loaded NPs were evaluated through various *in vitro* cellular studies. The results showed enhanced therapeutic efficacy of targeted drug loaded NPs over native drug and unconjugated NPs in HER 2 positive SKBR 3 breast cancer cell line. Moreover, exploration of the therapeutic benefits of rapamycin loaded Tmab conjugated NPs (Tmab-rapa-NPs) at molecular level, revealed augmented down regulation of mTOR signalling pathway thereby, inducing more cell death. Above all, our targeted multifunctional NPs have shown an excellent bio-imaging modality both in 2D monolayer and 3D tumor spheroid model. Thus, we can anticipate that such a multimodal nanotheranostic approach may be a useful tool for better cancer management in future.

* Corresponding author at: Nanomedicine Laboratory, Institute of Life Sciences, Nalco Square, Chandrasekharpur, Bhubaneswar, Orissa 751 023, India. Fax: +91 674 230072.
E-mail address: sanjeebsahoo2005@gmail.com (S.K. Sahoo).

http://dx.doi.org/10.1016/j.jcis.2015.03.049
0021-9797/

1. Introduction

Chemotherapeutic regimens are at the upper edge for cancer management; however nonspecific cytotoxicity, poor aqueous solubility and bioavailability become the major hindrance for the success of cancer treatment [1]. In this regard, efficient drug delivery approaches are the prerequisite for site specific delivery of chemotherapeutic agents by surmounting above limitations to enhance their therapeutic potential. Currently, research is mostly centred on development of suitable carrier systems for site specific delivery of therapeutics and numerous studies indicate that, these drug delivery vehicles are capable of overcoming the lacunas of current chemotherapeutic agents by enhancing their bioavailability, aqueous solubility and limiting toxicity [2–4]. Furthermore, improvement of cancer therapy also involves monitoring the therapeutic response of drug after treatment. In this setting, theranostic platforms are emerging as a combinational approach for simultaneous cancer diagnosis and therapy and nowadays much effort is focussed around development of such an approach for successful cancer management [5–7].

Rapamycin is a potent anticancer drug which is effectively used for the treatment of different cancers. The anticancer activity of rapamycin is attributed to its binding with the immunophilin FK506-binding protein (FKBP12) to form a ternary complex, which is capable of inhibiting the mammalian target of rapamycin (mTOR) – a serine/threonine kinase recognized as a central controller of eukaryotic cell growth and proliferation. The mTOR pathway is frequently activated in many human cancers, including breast cancer [8,9]. Inhibition of mTOR pathway by rapamycin blocks the phosphorylation of its downstream targets like the 70 kDa, 40S ribosomal protein kinase (p70S6K1) and the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) leading to G1 arrest in most cancer cells and p53-independent apoptosis in some others [10–13]. Therefore, inhibiting the mTOR pathway is extensively considered as an effective approach for targeted cancer therapy. Though rapamycin represent a potent anticancer drug in preclinical settings, its clinical utility is floundered due to its poor aqueous solubility, low bioavailability, non-specificity and dose limiting toxicity [14]. In this context, nano-drug delivery systems are anticipated to overcome the drawbacks associated with native rapamycin. In relation to this, numerous research groups including us have developed different nanoparticulate systems with sustained release property to surmount the shortcomings associated with native rapamycin [15–17]. Among the various nanotechnology based platforms, lipid based NPs are considered as one of the most promising drug delivery vehicle owing to their small particle size, ability to cross the biological barriers and for accumulating at the targeted site for efficient delivery of chemotherapeutic agents [18,19]. In this regard, glyceryl monooleate (GMO), an amphiphilic lipid molecule approved by the food and drug administration (FDA) is well explored to form lipid based NPs [20]. In general, GMO is known to form different liquid crystalline phases like reverse micellar phase, lamellar phase, cubic phase, reverse hexagonal phase etc. depending upon their water content [21,22]. It is also known to form a three dimensional network of curved lipid bilayers where both water soluble and insoluble drugs can be entrapped and explored for controlled drug delivery [23–25].

Primary requirement of a targeted cancer therapeutic approach is to deliver chemotherapeutic drugs to the cancer cells in a site specific manner over a period of time without affecting surrounding noncancerous tissues. In this setting, a monoclonal therapeutic antibody Trastuzumab (Tmab), which has been FDA approved can be used for targeted therapy against HER 2 positive breast cancer cells and thus can act as an attractive tumor targeting ligand [26,27]. Recently, optical imaging has been explored extensively in biomedical research and semiconductor nanocrystals known as quantum dots (QDs) have emerged as unique biological imaging and labelling probe due to its superior optical properties [28,29]. To accomplish superlative cancer therapy using nanotheranostic approaches, current researches mostly focus on using quantum dots in combination with anticancer agents to intermingle both therapy and imaging [30,31].

Thus, in the present investigation, with an aim to formulate a targeted nanotheranostic system for improved breast cancer therapy and imaging we developed a multifunctional lipid based NPs by using Tmab as targeting ligand, rapamycin as anticancer drug and quantum dots as imaging probe. To substantiate our hypothesis that the developed Tmab functionalized GMO based lipid NPs may exhibit higher uptake and therapeutic efficiency through HER 2 receptor mediated targeting, we have evaluated the efficacy of our formulated rapamycin loaded Tmab conjugated NPs (Tmab-rapa-NPs) in HER 2 positive and negative cell lines by cell cytotoxicity assay, uptake assay, apoptosis study, etc. The molecular mechanism related to apoptosis was investigated by western blot analysis. Further, the imaging potentiality of the targeted nanotheranostic system was validated in two dimensional (2D) monolayer culture and three dimensional (3D) tumor spheroid model *in vitro*. Thus, our preliminary studies suggest that such a theranostic nanocarrier may act as a multimodal vehicle for improved cancer therapeutics and imaging.

2. Materials and methods

2.1. Materials

Rapamycin was purchased from Fujian Kerui Pharmaceutical Co., LTD., Fuzhou City, China. Pluronic F-127, potassium bromide (KBr), Tween-80, propidium iodide (PI), 6-coumarin, protease inhibitor cocktail, sodium dodecyl sulfate (SDS), glycine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), p-Coumaric acid, luminol, glutaric acid, D-α-Tocopherol poly (ethylene glycol) 1000 succinate (TPGS), poly (ethylene glycol)-10,000 (PEG-10,000), N, N′-dicyclohexyl carbodiimide (DCC), N-hydroxy succinimide (NHS), uranyl acetate, Igepal CA-630 (NP-40), Sodium deoxycholate, Ethylene glycol-bis (2 amino ethyl ether)-N, N, N′,N′-tetra acetic acid (EGTA), Ethylene diamine tetra acetic acid (EDTA), agarose, DMSO-d_6 (99.9 atom% deuterium-enriched) and dimethyl sulphoxide (DMSO) were procured from Sigma–Aldrich (St. Louis, MO). Glyceryl monooleate (GMO) was obtained from Eastman (Memphis, TN). Sodium chloride was procured from MP biomedicals (Cedex, France). Skimmed milk powder was obtained from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Acetonitrile was purchased from Spectrochem, Pvt. Ltd. (Mumbai, India) and Tris base was obtained from Promega (Promega Corporation, Madison, Wisconsin). Mitotracker Red (CMXRos) dye and Qdot® 605 ITK™ amino (PEG) quantum dots (QD605) were purchased from Invitrogen Corp. (Carlsbad, CA). Trastuzumab (Tmab) was procured from Hoffmann-La Roche Ltd. (Basel, Switzerland).

2.2. Cell culture

All the cell culture experiments were performed by taking HER 2 positive SKBR 3 and HER 2 negative MDA-MB-231 breast cancer cell lines obtained from National Centre for Cell Sciences (NCCS), Pune, India and cultured using DMEM (PAN BIOTECH GmbH, Aidenbach, Germany) with 10% fetal bovine serum (FBS) supplemented with 1% L-glutamine and 1% penicillin – streptomycin (Himedia Laboratories Pvt. Ltd., Mumbai, India). The cells were