ELSEVIER

Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2018) xxx–xxx

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

A highly integrated precision nanomedicine strategy to target esophageal squamous cell cancer molecularly and physically

Xin-shuai Wang, PhD[a], Xue-zhen Ding, MD[a], Xiao-cen Li, PhD[b], Yixuan He, BS[b], De-jiu Kong, MD[a], Li Zhang, MD[a], Xiao-chen Hu, MD[a], Jun-qiang Yang, MD[a], Meng-qi Zhao, MD[a], She-gan Gao, PhD[a,*], Tzu-yin Lin, DVM, PhD[c,*], Yuanpei Li, PhD[b,*]

[a]*Henan Key Laboratory of Cancer Epigenetics; Cancer hospital, The First Affiliated Hospital, College of Clinical Medicine, Medical College of Henan University of Science and Technology, Luoyang, China*
[b]*Department of Biochemistry & Molecular Medicine, UC Davis Comprehensive Cancer Center, University of California Davis, Sacramento, CA, USA*
[c]*Department of Internal Medicine, UC Davis Comprehensive Cancer Center, University of California Davis, Sacramento, CA, USA*

Received 7 March 2018; accepted 13 June 2018

Abstract

The prognosis of esophageal squamous cell carcinoma is poor. We hereby presented a highly integrated and clinically relevant precision nanomedicine strategy to target ESCC molecularly and physically for significant improvement of the treatment efficacy. We firstly identified PI3K overexpression in patient samples and its relation to poor patient survival. With our highly versatile tumor-targeted drug delivery platform (DCM), we were able to load a potent but toxic docetaxel (DTX) and a PI3K inhibitor (AZD8186) with favorable physical properties. The combination of the DTX-DCM and AZD8186-DCM showed a highly efficacious and synergistic anti-tumor effect and decreased hematotoxicity. A pro-apoptotic protein, Bax was significantly upregulated in ESCC cells treated with combination therapy compared to that with monotherapy. This study utilized a highly integrated precision nano-medicine strategy that combines the identification of cancer molecular target from human patients, precision drug delivery and effective combination therapy for the development of better ESCC treatment.

Key words: Precision nanomedicine; PI3K inhibitor; Docetaxel; Esophageal squamous cell carcinoma; Nanoparticle

Esophageal squamous cell carcinoma (ESCC) remains significant health challenges around the world, with an estimated 482,300 newly diagnosed cases and 406,800 ESCC related deaths each year.[1] The incidence of ESCC is increasing, while the prognosis is extremely poor.[2] The 5-year survival rate of patients with esophageal cancer rarely exceeds 40%.[3] Local recurrence after initial treatment is the major cause of treatment failure in the patients.[4, 5] Treatment with curative intent involves either resection with or without neoadjuvant therapy, or definitive chemoradiotherapy with or without salvage resection.[6] The major limitation of chemotherapy includes a lack of specificity that results in low concentrations of chemotherapeutic drugs/agents at tumor sites, along with severe off-target toxic effects.[7] Therefore, there is an urgent critical need for new therapies and more effective strategies to enhance the curative effect and reduce the toxicities.

Molecularly targeted therapy has been an emerging trend for practicing precision medicine. Dysregulation of PI3K has been identified in several solid tumors, such as breast cancer, prostate cancer, including esophageal cancer.[8–11] However, the understanding of the role of PI3K in ESCC is limited. Basic molecular

Abbreviations: DCM, disulfide crosslinked-micelle; DTX, docetaxel; ESCC, Esophageal squamous cell carcinoma; IHC, immunohistochemistry; TEM, Transmission electron microscope; DLS, dynamic light scatting; CI, combination index; Fa, Fraction affected; FBS, Feutal bovine serum; PI, propidium iodide; DiD, 11,1′-Dioctadecyl-3,3,3′,3′-etramethylindotricarbocyanineiodide; OS, overall survival; AST, aspartate aminotransferase; ALT, alanine aminotransferase

Conflict of Interests: Dr. Li is the co-inventor of the patent for the cross-linked micelles and may have interest in commercialization. The remaining authors declare no competing financial interests. This work was supported by the NIH/NCI (R01CA199668), NIH/NICHD (R01HD086195), UC Davis Comprehensive Cancer Center Support Grant (CCSG) (P30 CA093373), CCSG Institutional Research Grants (IRG), and American Cancer Society IRG.

*Corresponding authors.
E-mail addresses: sggao2016@163.com, (S. Gao), tylin@ucdavis.edu, (T. Lin), lypli@ucdavis.edu. (Y. Li).

https://doi.org/10.1016/j.nano.2018.06.008
1549-9634/

biologists revealed that the suppression of esophageal tumor growth and chemoresistance could be achieved by targeting the PI3K/AKT pathway.[11–13] AZD8186, a novel small-molecule inhibitor of PI3Kβ and PI3Kδ inhibits the growth of tumor cells, regulating signaling through the PI3K/AKT signaling pathway.[14] AZD8186 is currently in the phase I clinical trial and already showed its promising anti-cancer activity as monotherapy or in combination with docetaxel (DTX) or androgen therapy against prostate cancer, breast cancer, T-cell acute lymphoblastic leukemia[15–17] DTX, a second-generation taxoid, is considered one of the most potent chemotherapeutic agents in the clinical setting[18] that is broadly indicated for the treatment of non-small cell lung cancer, breast cancer, ovary cancer, prostate cancer, stomach cancer, head and neck cancer and esophageal cancer.[19,20] However, similar to other lipophilic chemotherapy agents, it has major limitations including a lack of specificity that results in low concentrations of chemotherapeutic drugs/agents at tumor sites, along with severe off-target toxic effects.[7] Recently, to overcome these problems and improve anticancer effects, much attention has been focused on the design of nanometer drug delivery platform.

Nanotechnology-based drug delivery platforms offer several distinct advantages for cancer therapy,[21–23] such as improved drug solubility, minimal drug leakage during transit to the target, prevention of degradation and premature clearance of the drug, prolonged *in vivo* circulation time and preferential accumulation at tumor site *via* the enhanced permeability and retention (EPR) effect, biocompatibility and biodegradability of nanoplatforms. Our group had developed the reversible disulfide cross-linked micelles (DCMs)[24] that could minimize the premature release of drugs from carriers during circulation in the bloodstream and control the release rate of the entrapped drugs at the tumor sites.[25] We already demonstrated that DCM could greatly enhance chemotherapeutic drug efficacy and safety.[25] In this study, we introduced a highly integrated precision nanomedicine strategy that was able target ESCC at both molecular and physical level. We firstly identified the correlation between PI3K expression levels and ESCC patient survival. We then employed DCMs as a tumor-specific targeting strategy to deliver the molecular target inhibitor (AZD8186) and potent DTX to tumors in mouse model. Our study firstly demonstrated the synergistic effects *in vitro* and *in vivo* of AZD8186 and DTX within nanoformulations against ESCC and greatly reduced hematotoxicity.

Methods

Materials

DTX and AZD8186 were purchased from AK Scientific Inc. (Mountain View, CA, USA). 11,1′-Dioctadecyl-3,3,3′,3′-etramethylindotricarbocyanineiodide(DiD), a near-infrared dye, was purchased from Biotium (Invitrogen, USA). Annexin V and propidium iodide (PI) were obtained from (Pharmingen, San Diego, CA, USA). Esophageal cancer cell line KYSE70 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Antibodies for PI3K for IHC, pPI3KpAKT (T308), p53(DO-1), Bcl2, Bax (2D2) and β-actin were purchased from Millipore (Massachusetts, USA). Cholic acid, MTS [3-(4, 5-dimethyldiazol-2-yl)-2,5 diphenyl tetrazolium bromide] and all other chemicals were purchased from Sigma-Aldrich (St. Louis, USA) and used as received.

Table 1
The characteristics of patients and the PI3K expression level in esophageal cancer tissue.

Characteristics	Case (%)	PI3K expression (IHC)		
		high	middle	Low
Gender				
male	33 (55)	16	11	6
female	27 (45)	12	9	6
age (years)				
Median age	62			
≥60	32 (53)	17	10	5
<60	28 (47)	11	10	7
Lymphatic invasion				
yes	15 (25)	8	4	3
no	45 (75)	20	16	9
clinical stage				
I-II	44 (73)	21	16	7
III	16 (27)	7	4	5
Smoking status				
Non-smoker	36 (60)	18	14	4
Smoker	24 (40)	10	6	8
Alcohol consumption				
Non-drinker	52 (87)	27	18	7
Drinker	8 (13)	1	2	5

Immunohistochemistry

Expression of PI3K was examined using tumor samples (T) and para-tumor tissue (N) obtained from 60 patients who were diagnosed to have esophageal cancer and treated in the Department of surgical oncology, The First Affiliated Hospital of Henan University of Science and Technology between February 2012 and March 2014. The median age of the patients was 61 years, with a range of 42-79 years. The clinical characteristic of the patients are shown in Table 1.

Serial 3 μm thick sections from the formalin-fixed paraffin-embedded samples of esophageal cancer tissue were cut onto glass slides and advanced for immunohistochemical (IHC) staining. A standard immunohistochemical technique was performed using a Ventana BenchMark XT immunostainer (Ventana Medical Systems, Tucson, USA) with the PI3K antibody at a dilution of 1:200. Heat epitope retrieval provided by the immunostainer was done for 30 min. All slides were examined independently by two senior pathologists who were not informed of patients' clinical parameters. Every tumor was assessed by a score according to the predominant intensity of the cytoplasmic staining (no staining = 0, weak staining = 1, moderate staining = 2, strong staining = 3) and the extent of stained cells (0% = 0, 1-30% = 1, 30-60% = 2, 60-100% = 3). The scores ranged from 0 to 6. The score was 0-1, 2-3 and 4-6 indicating negative expression, middle expression, and positive expression, respectively.

Western blot and quantitative real-time PCR (qRT-PCR)

Protein samples were collected from tumor tissue and paired adjacent normal tissue of postoperative specimens from the 60