Cancer Letters 348 (2014) 177–184

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Anti-tumor efficacy of a therapeutic peptide based on thermo-responsive elastin-like polypeptide in combination with gemcitabine

Jung Su Ryu, Drazen Raucher *

Department of Biochemistry, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, United States

ARTICLE INFO

Article history:
Received 3 October 2013
Received in revised form 12 March 2014
Accepted 19 March 2014

Keywords:
Elastin-like polypeptide
Hyperthermia
p21
Anticancer drug delivery
Thermally targeting

ABSTRACT

This work describes the effects of elastin-like polypeptide (ELP) with the $p21^{Waf1/Cip1}$-derived cell cycle inhibitory peptide (p21) on pancreatic tumor cells with gemcitabine. The thermo-responsive property of ELP permits use of a mild, local hyperthermia to target tumors for the transport of chemotherapeutics. In this study, a p21-ELP construct with Bac cell penetrating peptide was designed, and its anticancer activities in pancreatic cancer cell lines was examined. In combination with gemcitabine, the peptide demonstrated enhanced *in vitro* cytotoxicity as well as tumor growth inhibition in an animal model. Our data suggest that this ELP construct, with gemcitabine, may improve pancreatic cancer therapy.

1. Introduction

Elastin-like polypeptides (ELP), derived from the mammalian tropo-elastin protein, consist of a repeated pentapeptide Val-Pro-Gly-Xaa-Gly (VPGXG) unit. In this unit, Xaa is a "guest residue" that can be any amino acid except Pro [1,2]. At some unique transition temperature Tt, ELP biopolymers undergo an inverse phase transition. Thus ELPs remain in solution at temperatures below Tt, but aggregate out of solution as Tt is surpassed. Just as importantly, a temperature decrease below Tt completely reverses the aggregation and insolubility [3–5].

We have previously reported our work in utilizing thermally responsive properties of ELPs to produce a transport system able to deliver anticancer therapeutic molecules into solid tumors [6–8]. This system consists of three components, a cell penetrating peptide (CPP) to promote the cellular uptake of an ELP, to which an anticancer cargo molecule can be attached (Fig. 1A). The overarching hypothesis here is that a systemically administered ELP aggregates and accumulates within a targeted tumor site upon localized, external applications of mild hyperthermia. By contrast, ELPs at physiological temperatures are expected to remain soluble and non-aggregated, clearing initially from non-heated tissues, and eventually from the system. By exploiting these thermal properties of ELPs, increased local concentrations of ELP–drug conjugates should be observed at heated sites, along with a prolonged construct residence time in thermally-targeted tumor regions. Hyperthermia itself may further increase the efficient delivery of ELP conjugates to tumor tissue via increased tumor vasculature permeability and perfusion [6]. That ELPs indeed accumulate at higher concentrations in heated versus unheated tumors has been verified in a number of anticancer drug delivery studies [9].

Moreover, our prior work has shown the feasibility of ELP/hyperthermia conjugated with cytotoxic small molecules like doxorubicin and paclitaxel [8–11] and therapeutic peptides such as c-myc inhibitory peptides and lactoferrin-derived peptide [12,13] as new cancer treatment modalities.

In our earlier study, ELP-p21 with Bac cell penetrating peptide on its N-terminus (Bac-ELP1-p21) have shown cell cycle inhibition and induced significant apoptosis in SKOV-3 ovarian cancer cell line [7]. However, this effort was hampered by its small yield size. To achieve a greater production yield for this ELP-p21 conjugate, we next reversed the polypeptide configuration to a p21-ELP_1-Bac configuration, with the p21 on the N-terminus of ELP and Bac on the C-terminus.

Pancreatic cancer, the fourth most common cause of death in United States, is annually diagnosed in three percent of all new

Abbreviations: ELP, elastin-like polypeptide; p21, $p21^{Waf1/Cip1}$-derived cell cycle inhibitory peptide; Tt, transition temperature; CPP, cell penetrating peptide; CI, combination index; CDK, cyclin dependent kinase.

* Corresponding author. Tel.: +1 601 984 1510; fax: +1 601 984 1501.
E-mail address: draucher@umc.edu (D. Raucher).

http://dx.doi.org/10.1016/j.canlet.2014.03.021
0304-3835/

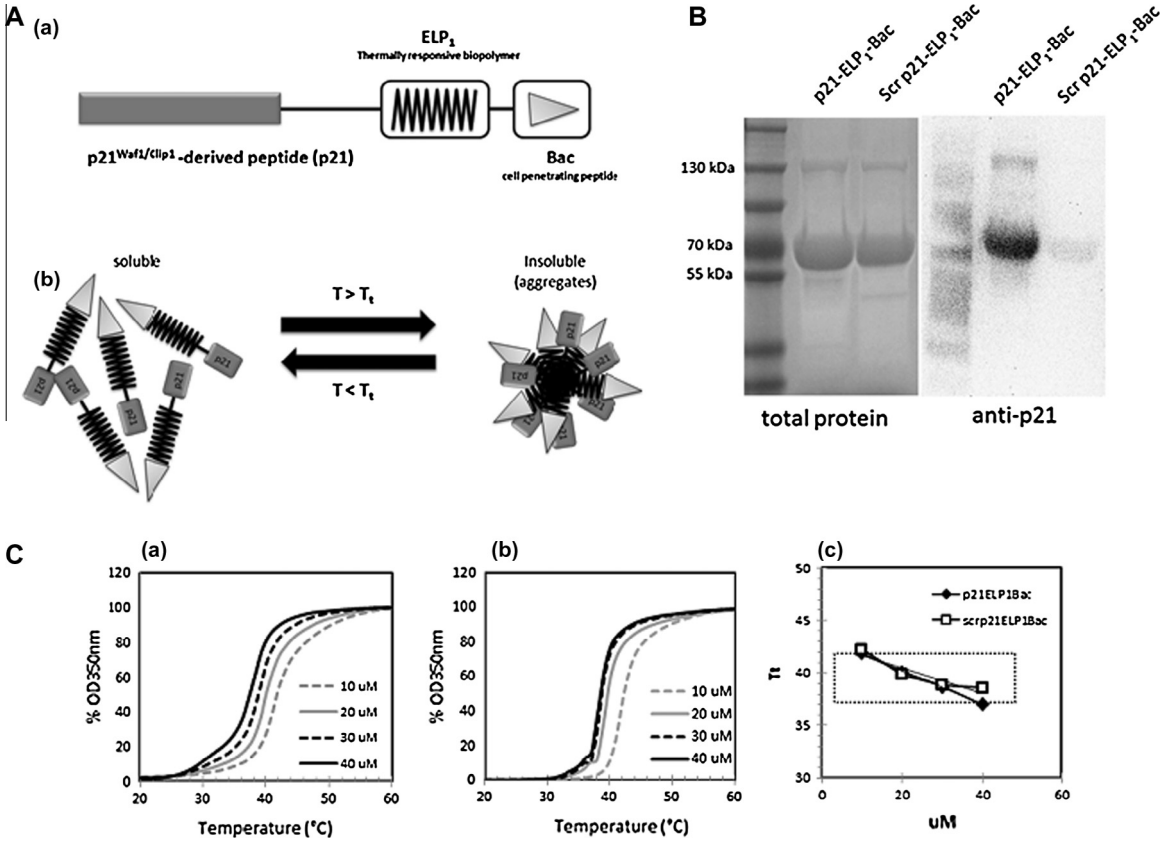

Fig. 1. (A) Schematic diagrams of an ELP-CPP drug delivery system. (a) The delivery system consists of the p21, an anticancer cargo molecule at the N-terminus, followed by the thermally responsive elastin-like polypeptide and Bac cell penetrating peptide. (b) Thermally responsive property of ELP. Aggregation of ELP at high temperature (T > Tt) can increase of the tumor accumulation of anticancer cargo molecule, p21. (B) Expression of p21-ELP1-Bac in *E. coli*. 1 μg of each ELP conjugates were loaded onto SDS-gel. Protein staining (total protein) showed that each ELP conjugate has a molecular weight of 62 kDa and no impurity issues. Western blot analysis also revealed that p21-ELP conjugate and its scrambled control protein were well-expressed in *E. coli*, as we designed. (C) Thermal characterization of p21-ELP_1-Bac and its scrambled control. The temperature-induced aggregation of (a) p21-ELP_1-Bac and (b) scr p21-ELP_1-Bac was characterized in various concentrations (10–40 μM). (c) The Tt was defined as the temperature at which the Abs350 reached 50% of the maximum turbidity. Dotted line represents clinically applicable Tt (37–42 °C).

cancer cases, the same percentage at which pancreatic cancer patients die from their disease [14]. The 5-year survival rate with pancreatic cancer is less than 10%, making it one of the most fatal cancers. Gemcitabine, the first line of treatment for pancreatic cancer, is of limited use owing myelo-suppression [15]. A number of efforts are currently underway to combine gemcitabine with other drugs either cytotoxic and/or biological targeted compounds. There have not been any outstanding treatment options than gemcitabine for pancreatic cancer. An urgent need in the contemporary cancer clinic thus exists for novel approaches that can be specifically targeted to pancreatic cells.

Here, we applied this ELP/hyperthermia strategy using our newly developed Bac cell penetrating peptide and $p21^{Waf1/Clip1}$-derived peptide, p21-ELP_1-Bac, within both *in vitro* and *in vivo* studies of three pancreatic cancer cell lines. The feasibility of this ELP/hyperthermic cancer treatment modality for treating pancreatic cancer was examined, and the effectiveness of this combination therapy with gemcitabine was also assessed.

2. Materials and methods

2.1. Design of construct and protein purification

All constructs were designed and synthesized as described previously [2,4]. Briefly, an elastin-like polypeptide coding sequence was modified by adding a Bac cell penetrating peptide (RRIRPRPPRLPRPRPRPLPFPRPG) to the ELP's C terminus, then further adding a $p21^{Waf1/Cip1}$-derived peptide (GRKRRQTSMTDFYHSKRRLIFSKRKP, p21) or its scrambled control (RTDKIKFRKFLRSRRSQP-MYSTKRGH) to the NH_2 terminus. For this study ELP_1 represents the elastin-like polypeptide having 150 repeats of VPGXG with guest residues (amino acid at position X) Val, Gly, and Ala in a 5:3:2. After the resulting sequence had been confirmed by DNA sequencing, both constructs were expressed in *Escherichia coli* strain BLR(DE3) using a pET 25b expression vector, purified by repeated inverse transition cycling, and checked by SDS-PAGE and western blot analysis for their purity and molecular weights and their expressions of p21, respectively.

2.2. Characterization of transition temperature

Temperature-induced aggregations of the p21-ELP-bac and its scrambled control protein were characterized by a temperature-controlled, UV–visible spectrophotometer (Cary 100, Varian instruments). The p21-ELP_1 conjugates at various concentrations were analyzed in Dulbecco's Modified Eagles Medium (Cellgro, Manassas, VA) with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), monitoring absorbance at 350 nm (A_{350}) as a function of temperature at a constant rate (1 °C/min). The Tt was defined as the temperature at which A_{350} reached 50% of maximum turbidity.

2.3. Cell culture and animals

All animal protocols were approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee (IACUC). The human pancreatic cancer cell line S2013 was a generous gift from Dr. Lacey McNally at the University of Alabama at Birmingham; Mia PaCa-2 and Panc-1 were obtained from ATCC (Rockville, MD, USA). All cell lines were grown and maintained at 37 °C, 5% CO_2 in Dulbecco's Modified Eagles Medium with 10% fetal bovine serum. For animal studies, female athymic nude mice (Ncr-nu/nu), purchased from the National Cancer Institute (NCI, Frederick, MD), were housed in a specific pathogen-free condition with 12-h day/night schedule and fed with sterile food and R/O water ad libitum.