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Short Communication

The new ApoE analog DPT-Cog inhibits PI3k/Akt-dependent survival of human radio-resistant U87G glioblastoma cells

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ABSTRACT

Numerous human cancers including radio-resistant human glioblastomas require a constitutive activation of the PI3K-Akt pathway for their survival. In this context the protein phosphatase-2A (PP2A) family of ser/thr protein phosphatases is a central regulator of cell homeostasis that counteracts the aberrant oncogenic PI3K survival signal. Importantly the trimeric ABαC holoenzyme, named $PP2A_1$, specifically counteracts the aberrant oncogenic constitutively activated PI3K survival signal. In this regard we recently reported that the pharmacological activation of PP2A mediated by the sphingolipid analog FTY720 down-regulated the constitutively active PI3K/Akt pathway involved in the survival of radio-resistant U87G human glioblastoma cells. In this study, using a peptide-based drug phosphatase technology (DPT) we rational designed a new PP2A activator corresponding to an ApoE-mimetic bipartite-peptide, named DPT-Cog, that combines the cellular inactive DPT-sh1 shuttle (VKKKKIKREIKI) and the PP2A activating COG133 sequence (LRVRLASHLRK LRKRLL). We first demonstrated that this new chimeric DPT-sequence down-regulated the PI3K-Akt survival pathway and inhibited the survival of U87G cells. In addition we found that DPT-Cog decreased the growth of human glioblastoma U87G cells. Furthermore, DPT-Cog also decreased the growth of X-irradiated U87G senescent cells. In conclusion we characterized the DPT-Cog molecule, a new ApoE cog-mimetic and potential anti-tumor peptide. Our results clearly indicate that DPT-Cog counteracted PI3K survival pathways of U87G cells and is also toxic against irradiated U87G senescent cells.

KEYWORDS: radioresistance, glioblastoma, DPT-Cog

INTRODUCTION

Protein phosphatase-2A (PP2A) is a major family of ser/thr protein phosphatases comprising multiple holoenzymes that are made of a core dimer, composed of a catalytic (C) subunit and a structural (A) subunit, in combination with a third variable regulatory (B) subunit that determines substrate specificity, subcellular localization and enzymatic activity of a defined holoenzyme [1]. Constitutive activity of the PI3K-Akt pathway and induction of cellular senescence are often associated with resistance to cancer therapies [2, 3]. Multiple studies have also established that PP2A is a tumor suppressor frequently inhibited in human tumors and represents a druggable target against cancer [4]. Interestingly, the inhibition of the trimeric ABαC PP2A holoenzyme named $PP2A_1$, can activate tumor survival such as Akt, β-catenin and c-Myc that are associated with cancer progression [5-8]. $PP2A_1$ inhibition can also activate the specific neurodegenerative pathway resulting from hyperphosphorylation of the Tau protein [9, 10]. Pro-tumoral and pro-degenerative inhibitory effects mediated by PP2A can result from genetic mutation(s) or an up-regulation of endogenous inhibitors [4, 9]. In this regard Apolipoprotein E (ApoE) is a plasma protein responsible for

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transporting lipid and cholesterol. ApoE and ApoE-mimetic peptides bind to the PP2A inhibitor SET to activate endogenous PP2A activity [10, 11]. The use of ApoE mimetic peptides were recently proposed as an innovative approach for the treatment of human pancreatic cancer that usually or frequently overexpressed specific PP2A inhibitors such as SET or CIP2A [12]. It has been shown that Cog133, a sequence corresponding to the amino acid residues 133-149 of the ApoE receptor binding region [13, 14], binds to PP2A inhibitor SET and results in PP2A activation.

We have recently demonstrated [15] that, in contrast to non tumor DHF fibroblasts, the PI3K pathway is activated in human U87G and SF763 glioblastoma cells that constitutively express phosphorylated Akt (pSer473). We also found that two pharmacological inhibitors Ly 294002, a PI3K inhibitor, and A6730, an Akt/PKB inhibitor inhibited the survival of U87G glioblastoma cells and down-regulated the constitutive activation of phosphorylated Akt (ser-473). In addition we recently characterized [15] a senescent-like phenotype in U87G cells mediated either by PP2A pharmacological inhibition with okadaic acid or by X-irradiation (2 Gy) as previously reported by others [16].

To specifically deregulate PP2A-directed intracellular pathways we previously characterized an inactive shuttle, named DPT-sh1 [17], that we used to develop the drug phosphatase technology (DPT) concept involving the design of PP2A-interacting pro-apoptotic cell-penetrating peptides named DPT-peptides [17-20]. In this study we tested the potential anti-tumor properties of DPT-Cog in radio-resistant U87G cells. We found that DPT-Cog counteracted the constitutive activation of the PI3K pathway and also inhibited U87G cell survival. In addition we found that DPT-Cog remained toxic against irradiated U87G cells that expressed SA-β-beta-gal senescence biomarker.

MATERIALS AND METHODS

Cells

We used the highly radio-resistant U87G glioblastoma cells (kindly gifted by Pr. Marie Dutreix, Curie Institute, Orsay). The cells were maintained in Dulbecco's modified Eagle's medium (Gibco) with 10% fetal bovine serum, without any (antibiotic and incubated at 37 °C in a humidified atmosphere of 5% CO_2.

Pharmacological agents

PP2A activator FTY720 was obtained from Euromedex. For peptide synthesis, high-performance liquid chromatography-purified NH2–biotinylated peptide (purchased from Proteogenix) was prepared by solid-phase peptide synthesis, dissolved in dimethyl sulfoxide (DMSO) and stored at -20 °C for later use.

Radiation treatments

Radiation and the measurement of cell density including ICW Image Processing were performed as previously described [15].

Cytotoxicity assays

A total of 3,000 cells were incubated for 24 hours with different concentrations of pharmacological agents. Cell cytotoxicity was analyzed by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (called MTT) for adherent cells as described by the manufacturer (Sigma).

Western blot analysis

Exponentially growing cells (10^5 cells) were seeded overnight in 24-well culture cell plate, at sub confluent monolayer, prior to pharmacological treatments. Cell extracts were prepared as previously described [15], and the protein concentration in each sample extract was quantified using Bio-Protein assay Bio-Rad Laboratories. Lanes were loaded with the material corresponding to 20-40 µg of cell protein extract. The following antibodies were used: Phospho-AKT (Ser 473) (D9E) rabbit mAB, 1/2000 and AKT (pan) (C67E7) rabbit mAB, 1/2000 obtained from Cell Signaling Technology, and HP1γ (2MOD-1G6) mouse mAB, 1/5000 from Euromedex. In addition goat peroxydase labeled anti-rabbit IgG (H+L), PI-1000 or horse peroxydase labeled anti-mouse IgG (H+L), PI-2000 from VECTOR Laboratories was used as the secondary antibody. Immunoreactivities were visualized using PIERCE ECL western blotting substrate, the MY ECL IMAGER (Thermo Scientific) and the software ImageJ 1.45s (National Institutes of Health, USA; http://imagej.nih.gov/ij).

RESULTS AND DISCUSSION

Surgery is the major treatment of glioblastoma followed by concurrent chemotherapy and radiotherapy. Importantly radiation tolerance remains a major problem for efficient therapy. In this regard, as described in reference [15], we recently demonstrated that U87G glioblastoma cells are resistant to X-ray radiation at 2 Gy (100% survival). Interestingly, even at higher irradiation rates (10 Gy) U87G cells appeared highly resistant (85% survival) [15].

Importantly a constitutive activity of the PI3K-Akt pathway and induction of cellular senescence are often associated with resistance to cancer therapies [21, 22]. In this regard we recently demonstrated that the PP2A activator immuno-suppressant FTY720 inhibited a constitutively active PI3K-Akt survival pathway and counteracted radiation-induced senescence involved in radio resistance of U87G glioblastoma cells [15].

A previous work established that, the fusion of COG133 with the protein transduction domain derived from the Drosophila antennapedia generated a bipartite cell-penetrating peptide named COG112 that enhances COG133 bioactivity [23]. In this regard, hereby, we apply the DPT-technology to generate a new, powerful ApoE mimetic DPT-peptide named DPT-Cog, resulting from the fusion of COG133 with penetrating DPT-sh1 sequence (see table 1 for sequence details). We studied the toxic effects of DPT-Cog on Akt activity, and the survival of radio-resistant U87G glioblastoma cells. In addition we also monitored the effects of DPT-Cog on radio-senescent U87G cells.

DPT-Cog inhibits PI3K-dependent survival of radio-resistant U87G cells

Using MTT assay we first examined the cytotoxic effect of DPT-Cog, described in table 1, on the survival of U87G glioblastoma cell line. We found that 24-hr treatment of U87G cells with increasing concentrations of DPT-Cog resulted in a dose-dependent reduction of cell viability with an estimated IC_{50} inhibition rate of 25 μM (Fig. 1A). In addition, consistent with previously described cell survival dependence mediated by a constitutive activity of PI3K/Akt pathway [15], western blot analyses showed that DPT-Cog also decreased Akt phosphorylation (ser473) of U87G glioblastoma cells (Fig. 1B). Together these results indicated that DPT-Cog counteracts a constitutively active PI3K/Akt pathway that is required for the survival of U87G glioblastoma cells.

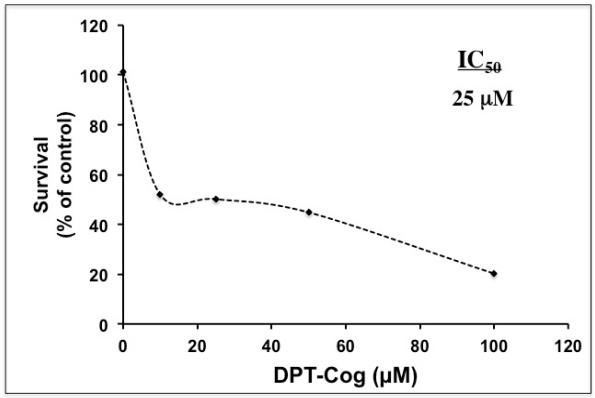
DPT-Cog inhibits growth of non irradiated or irradiated human U87G glioblastaoma cells

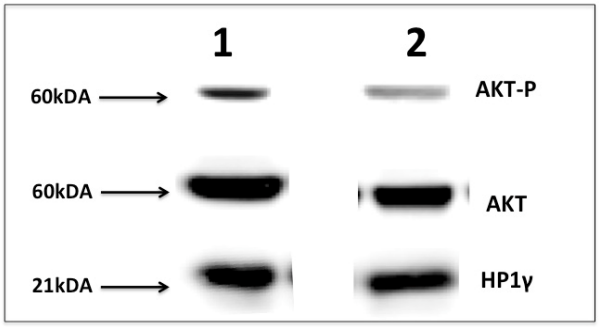
We recently reported that irradiated (2Gy) U87G cells expressed the SA-β-Gal senescence marker [15]. In addition we have also reported that PP2A activation, induced by FTY720 treatment, strongly reduced the percentage of irradiated U87G senescent cells expressing SA-β-Gal senescence marker and inhibited cell growth. In this study, as illustrated in fig. 2A, treatment with increasing concentrations of DPT-Cog for 72 hrs resulted in a dose-dependent reduction of cell density in the U87G glioblastoma cells. Furthermore, similar inhibitory effects were observed in U87G cells expressing SA-β-Gal senescence marker induced by X-ray radiation (2Gy) [15] and cultured 4 days prior to DPT-Cog treatment (Fig. 2B). Together our results illustrated the inhibitory effects of DPT-Cog on the constitutive activity of PI3K pathway, cell survival and growth of non irradiated or X-ray radiation induced cell senescence of U87G glioblastoma. These results also suggest that the propensity to senescence of radio-resistant U87G glioblastoma cells [15, 16] cannot antagonize DPT-mediated inhibitory growth effects.

Table 1. Origin, acronym and sequence of DPT sequences.

Protein of origin	Acronym	Sequence
CK2α	DPT-sh1	VKKKKIKREIKI
ApoE	Cog133	LRVRLASHLRKLRKRLL
CK2α + ApoE	DPT-Cog	VKKKKIKREIKI LRVRLASHLRKLRKRLL

A single letter amino acid code is used for all peptides.



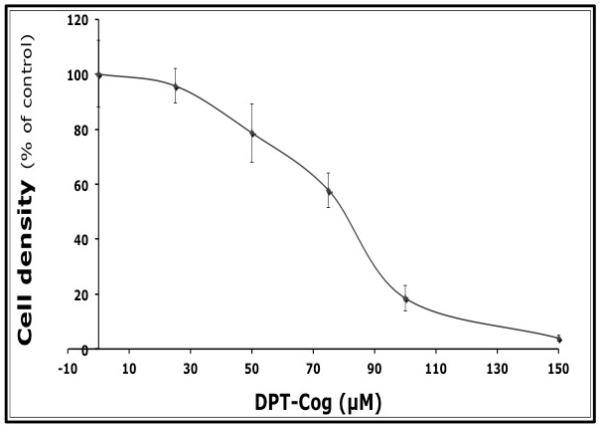


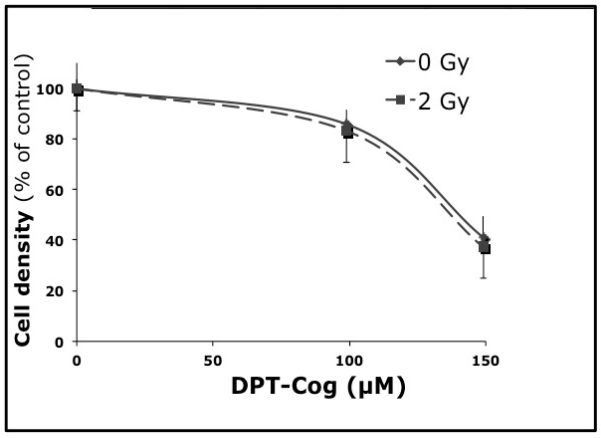
A **B**

Fig. 1. DPT-Cog inhibits a constitutively active PI3K/Akt survival pathway in U87G and SF763 cells.

(A) U87G cells were treated for 24 h with various concentrations of DPT-Cog and cell viability was analyzed by MTT test (n = 3). Ic50 survival inhibition rate mediated by DPT-Cog was estimated according to the inhibition curve of cell viability.

(B) U87G cells were treated for 5 h with DPT-Cog (1 = Control, 2 = 150 µM) and the effects of DPT-Cog on PI3K/Akt survival pathway were assessed by western blot Akt-phosphorylation using a specific Akt473 antibody analysis. The same blot was re-probed with a monoclonal antibody to total Akt and with an antibody to HP1γ as internal control.





A **B**

Fig. 2. Effect of DPT-Cog pharmacological treatment on non-irradiated and X-ray irradiated cells on cell growth.

(A) Cell growth curves of U87G cells after DPT-Cog treatment: U87G cells were seeded at 2 x 10^4 cells per well in 48-well microplates and treated with different doses of DPT-Cog (0-150 µM). Cell densities were recorded according to the DPT-Cog concentration indicated at 72 hours after drug treatment; SD is shown (n = 4).

(B) Cell growth curves of U87G cells following exposure to X-ray radiation and DPT-Cog treatment: U87G cells were seeded at 10^4 cells per well in microplates and 18 hours later the cultures were irradiated (0 and 2 Gy). The cell densities in culture of X-irradiated cells (0 and 2 Gy) cultured for 4 days prior to treatment with DPT-Cog for 24 h were recorded according to the indicated DPT-Cog concentration (0-150 µM), (n = 4).

CONCLUSION

We demonstrated that the new ApoE cog-mimetic, named DPT-Cog inhibited PI3K-dependent survival of non-irradiated and also irradiated radio-senescent U87G cells. Our results clearly indicate that the DPT-Cog alone or combined with irradiation could be clinically evaluated as a potential therapeutic molecule against PI3K-dependent human radio-resistant and radio-senescent glioblastomas.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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