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A synthetic cantharidin analog for the enhancement of doxorubicin suppression of stem cell-derived aggressive sarcoma

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ABSTRACT

Failure to cure many cancers once they are disseminated has been attributed to the presence of resistant cancer stem cells. Cantharidin, a natural compound isolated from the beetles and other insects has been traditionally used as anticancer agent, but limited by its significant toxicity. It has shown that cantharidin can force cancer cells prematurely into cell cycle and subsequently induce apoptotic cell death through the inhibition of protein phosphatase 2A (PP2A). In this study, we showed that a synthesized analog of cantharidin, LB1, with significant PP2A inhibition activity but without apparent toxicity, greatly enhanced the effectiveness of the standard anti-sarcoma chemotherapeutic agent, doxorubicin (DOX), in the xenograft growth inhibition and lung metastases prevention of an aggressive sarcoma derived from transformed mesenchymal stem cells in syngeneic rats. We report here on the possibility of, pharmacologic inhibition of PP2A with low toxicity cantharidin derivatives may be a useful strategy to enhance the effectiveness of DNA-damaged chemotherapeutic drugs against stem cell-derived cancer.

1. Introduction

Cantharidin, a natural compound isolated from the beetles and other insects with the inhibition activity of protein phosphatase 2A (PP2A), has been traditionally used as an anticancer agent for a long time [1,2]. However, the clinical application of cantharidin is limited due to its severe side-effects and highly toxic nature. Therefore, it is an urgent need to develop more selective and effective analogs of cantharidin with less toxicity for cancer treatment. Very recently, LB1.2, a cantharidin derivative with no evidence of acute or chronic toxicity has been synthesized and shown a significant enhancement of cancer chemotherapy on glioblastoma multiforme and neuroblastoma cancer cells by driving quiescent cancer cells into cycle and by blocking other replication checkpoints triggered by DNA damage through the significant inhibition effect of PP2A [3].

The failure of chemotherapy has been attributed to the presence of resistant cancer stem cells or, at least, cells in a dormant state, and in part, to the induction of defense mechanisms that protect both normal and cancer cells from cytotoxic injury [4–6]. Sarcomas are malignant mesenchymal tumors believed to be derived from transformed mesenchyal stem cells [7–11]. Despite improvements in treatment of primary sarcomas, many patients present with locally advanced or metastatic disease attributed to the presence of resistant cancer stem cells or cells in a dormant state, for which systemic treatment is largely unsuccessful [12–15]. It is interesting to test if inhibition of PP2A by cantharidin derivatives will be useful to the treatment of stem cell-derived cancers by enhancing the effectiveness of cytotoxic chemotherapeutic drugs.

In the present study, we have established an aggressive sarcoma model derived from rat transformed mesenchymal stem cells (rTDMCs), which can give rise to tumors as xenografts in syngeneic rats resembling fibrosarcomas that metastasize to the lungs but not to other organs, similar to some human sarcomas [16]. Like human sarcomas, these rat xenogratfts are only modestly sensitive to the standard anti-sarcoma chemotherapeutic agent, DOX, potentially because of their stem cell-like tendency to remain relatively dormant. In this work, we aimed to investigate the potential enhancement

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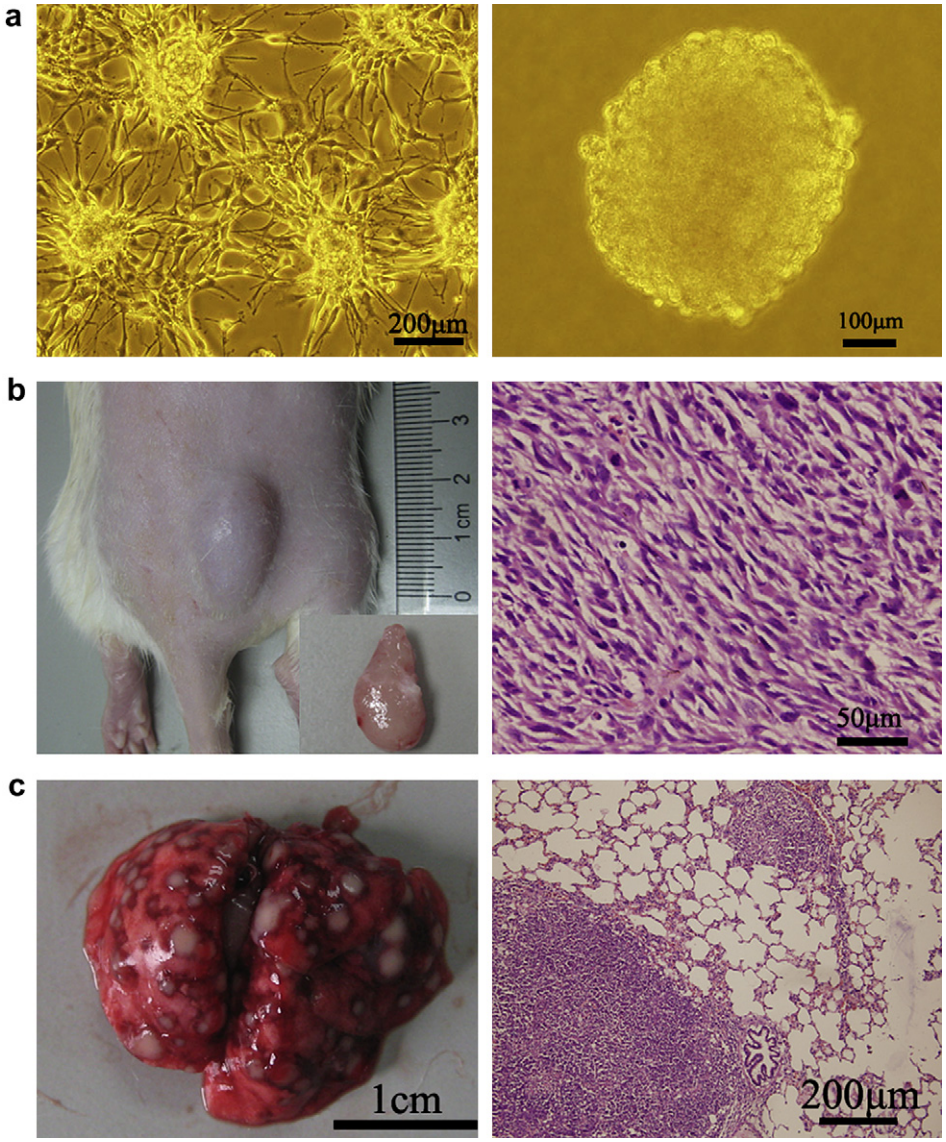

Fig. 1. Characteristics of the rTDMC cell line. (a) Morphology of rTDMC cells and colony formation of rTDMC cells in soft agar(arrow) (b) s.c. tumor mass 2 weeks after implantation of 5×10^6 cells and an excised s.c. tumor (left) and hematoxylin–eosin (HE) stained tumor tissue showing a storiform pattern with pleomorphic fibroblastic, histiocytic, and (rarely) multinucleated giant cells (right). (c) Multiple pulmonary metastases (left) and HE stained section of a metastasis (right).

effect of the cantharidin analog LB1, a water soluble homolog of LB1.2, on the effectiveness of DOX against this aggressive sarcoma derived from stem cells.

2. Materials and methods

2.1. Materials

LB1, a cantharidin analog and small molecule inhibitor of PP2A, was provided by Lixte Biotechnology Holdings, Inc (NY, USA). LB1 is a water soluble homolog of LB1.2 (4-(3-carboxy-7-oxa-bicyclo [2.2.1] heptane-2-carbonyl) piperazine -1- carboxylic acid tertbutyl ester) as reported previously [3]. Doxorubicin (DOX) was purchased from Main Luck Pharmaceuticals, Inc (Guangdong, China). IMDM medium and Fetal bovine serum (FBS) were from Gibco (NY, USA). Antibodies for immunofluorescence staining include anti-vimentin, anti-S-100, anti-alpha-smooth muscle actin (α-SMA) and anti-PCNA proteins were from BOSTER (Hubei, China). Rat-myosin heavy chain (MHC) and rabbit polyclonal antibody against tubulin were from Santa Cruz (CA, USA). FITC, Cy3-conjugated goat anti-mouse IgG and DAPI were from Beyotime (Shanghai, China). Chemicals for induced differentiation include β-glycerophosphate, dexamethasone, ascorbate, indomethacin, insulin, 3-isobutyl-L-methylxanthine and 5-Aza were all from Sigma (St. Louis, USA). For western blot, antibodies include phosphospecific antibodies against p–p53 (Ser15), p-ERK1, p-MdM2, p-Akt, and antibodies against total Akt and ERK1 were all from Cell Signaling Technology (MA, USA).

2.2. Cell culture

We derived a subclone of transformed dermis-derived mesenchymal stem cells (rTDMCs) from neonatal rat skin dermis [17]. Cells were cultured in IMDM medium supplemented with 10% FBS and incubated at 37 °C with 5% CO_2. At 80% confluence, cells were split 1:3 and cultured for one passage.

2.3. Soft agar assay

Colony formation by rDMC and rTDMC cells was assessed in soft agar, according to the method of Wada [18]. In brief, 500 cells were suspended in 1 ml of IMDM media with 10% FBS and 0.3% agar and layered over 0.6% agar IMDM media with 10% FBS. Colonies with more than 30 cells were counted 14 days after plating.

2.4. Tumorigenicity of rTDMC in syngeneic rats

Three- to 4-week-old male Sprague–Dawley (SD) rats, weighing 70-90g, were purchased from the laboratory animal center of the Third Military Medical University. Animal protocols were in accordance with the "Animal Care and Use Committee Guidelines of the Third Military Medical University". Tumorigenicity