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Inhibition of isoprenylcysteine carboxylmethyltransferase sensitizes common chemotherapies in cervical cancer via Ras-dependent pathway

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ABSTRACT

Isoprenylcysteine carboxylmethyltransferase (Icmt) catalyzes the last step of post-translational protein prenylation, which is essential for the stability and proper functions of many oncogenic proteins, such as Ras. Despite extensive studies on the roles of Icmt in tumor transformation and progression, little is known on the involvement ofIcmt in the development of tumor resistance to chemotherapy. Here we show the upregulation of Icmt as a persistent response to chemotherapy in cervical cancer cells. In-depth functional analysis demonstrated that Icmt inhibition significantly inhibited growth, induced apoptosis and augmented the inhibitory effects of chemotherapy drugs in cervical cancer in cell culture system and xenograft mouse model. Importantly, combination of Icmt specific inhibitor cysmethynil with doxorubicin or paclitaxel at sublethal concentration achieved almost full inhibition of tumor cell growth and survival. The remarkable synergy between chemotherapy drugs and Icmt inhibition in cervical cancer cells is likely due to the additional suppression of Ras and its downstream signaling pathways. We are the first to demonstrate the contribution of Icmt in tumor cells in response to chemotherapy. Our work also highlights Icmt inhibition as a sensitizing strategy for the treatment of cervical cancer or other Ras-driven tumors.

1. Introduction

Cervical cancer is the second most common cause of cancer deaths for women worldwide [1]. Current therapies for advanced or recurrent cervical cancer are platinum based chemotherapy [2]. However, the majority of the patients develop chemoresistance and relapse quickly [3]. Substantial advances have been made in the understanding of cervical cancer transformation and development, such as human papilloma virus (HPV) infection, oncogenic activation and upregulated mitochondrial metabolism [4–7]. The mechanisms underlying persistence of cervical cancer cell in response to chemotherapy have yet remained unknown and are important for the identification of sensitizing strategy for cervical cancer treatment.

Isoprenylcysteine carboxylmethyltransferase (Icmt) is an enzyme which catalyzes the last of the three-step post-translational protein prenylation [8]. Prenylation process serve as a mediator of subcellular localization or a determinant for protein stability, which plays essential roles in the proper functions of many oncogenic proteins, such as Ras [9,10]. Ras is mislocalized, and fails to transform fibroblasts and promote myeloproliferative disease in cells that lack Icmt [11,12]. Extensive studies demonstrate that inhibition of Icmt impairs growth and induces death of various type of cancer cells [13–16], making this enzyme as a promising cancer target. Besides regulating Ras functions in cancer cells, other roles of Icmt has been recognized, including regulating mitochondrial respiration and cancer cell metabolism [8,17].

In this study, we investigated whether Icmt is involved in cervical cancer cell response to chemotherapy. We showed that Icmt mRNA and protein levels were increased in cervical cancer cells after exposure to standard chemotherapy agents. We further showed that Icmt inhibition via specific pharmacological inhibitor or siRNA significantly sensitized cervical cancer cells to chemotherapy in vitro and in vivo, via a Ras-dependent pathway.

2. Materials and methods

2.1. Cell cultures and generation of cell lines

Human cervical cell lines SiHa (ATCC HTB-35) and C-33A (ATCC HTB-31) were cultured in Eagle's Minimum Essential Medium (Lonza,

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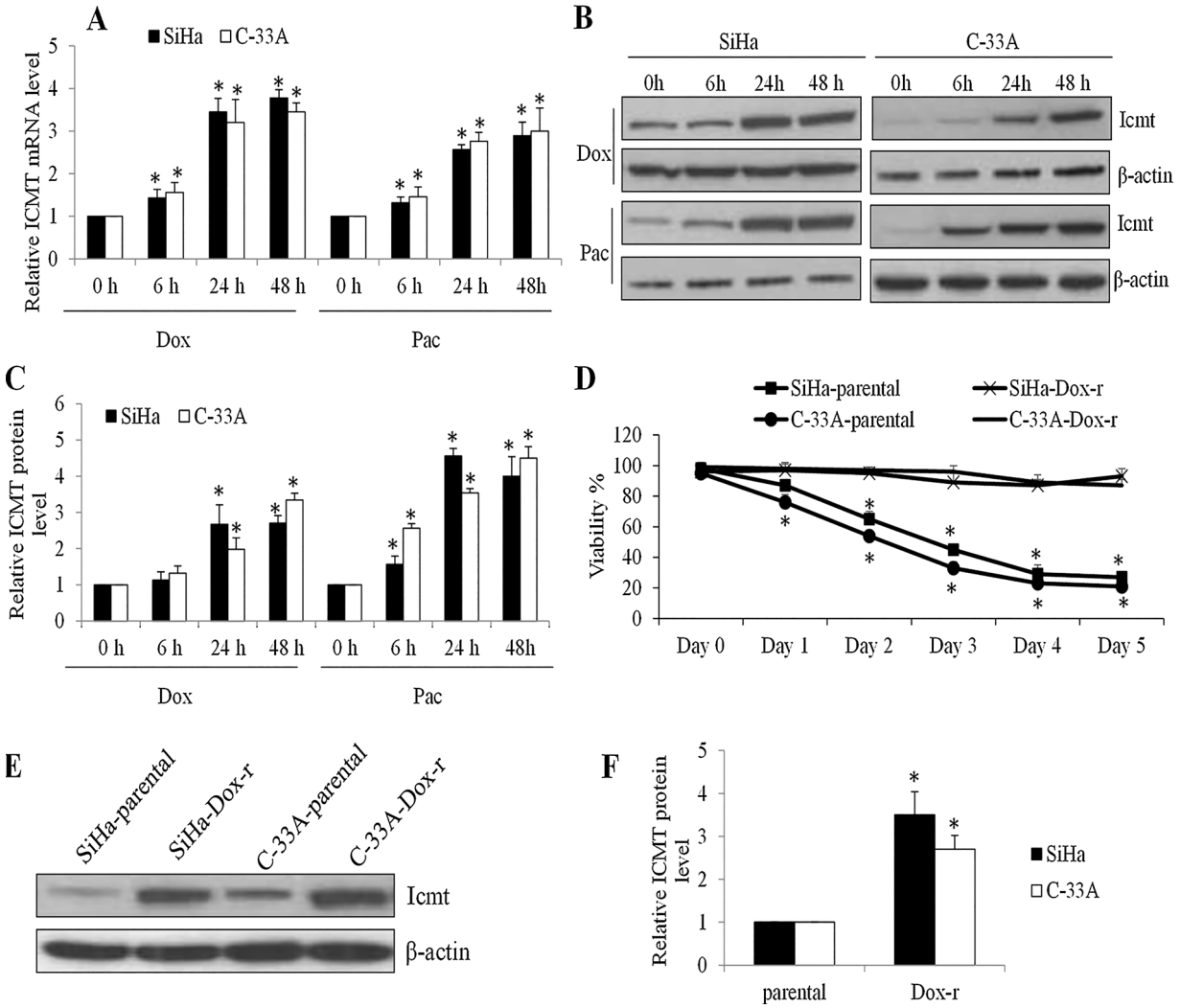

Fig. 1. Chemotherapy increases Icmt levels in cervical cancer cells.Chemotherapeutic agents doxorubicin (Dox) and paclitaxel (Pac) significantly increases Icmt's mRNA (A) and protein (B and C) levels in SiHa and C-33A cells in a time-dependent manner. 1 μM of paclitaxel and 50 nM of doxorubicin was used for time course analysis. (D) DMSO (control) and 1 μM doxorubicin-treated viable cells were determined at indicated time points by flow cytometry of Annexin V/7AAD staining. Annexin V-negative/7AAD-negative were counted as viable cells. (E and F) Icmt protein level is increased in doxorubicin-resistant SiHa and C-33A cells. Quantification of western blot bands' density was performed using Image J software. Results were presented as relative to control. Data were mean of three independent experiments (N = 3). $^{*}p < 0.05$, compared to control.

US) supplemented with 10% fetal bovine serum (Invitrogen, US). SiHa-Dox-r and C-33A-Dox-r cells were derived from each original parental cell line by continuous exposure to doxorubicin (Sigma, US; Cat. No. D1515). Initially, cells were treated with doxorubicin at 10 nM for 3 days. The media was removed and cells were allowed to recover for 3 days. The concentrations were gradually increased to 1 μM and the development period was carried out 8 months. Resistant cells were then maintained continuously in the presence of doxorubicin.

2.2. Cellular assays

Cells were treated with doxorubicin, cysmethynil (Cayman Chemical, US; Cat. No. CAS851636-83-4), paclitaxel (Sigma, US; Cat. No. T7402) or combination of cysmethynil and doxorubicin or paclitaxel for 3 days. Cell proliferation and apoptosis assays were carried out using the same protocol reported in our previous studies [18]

2.3. Western blot analyses

Total protein was extracted from cells using ice-cold RIPA buffer supplemented with protease inhibitor cocktail (Roche Diagnostics, France). Protein concentrations were determined using the bicinchoninic acid assay. Equal amount of proteins were separated by denatured SDS-PAGE gel, transferred to polyvinylidene difluoride membranes (BioRad, US) and then analysed by western blot. Antibodies against p-Raf at Ser338 (Abcam, Cat. No. ab135559), p-ERK at Thr 202/Tyr 204 (Santa Cruz, Cat.No. sc-16982), p-Akt at Ser437 (Sant Cruz, Cat. No. sc-7985-R), p-mTOR at Ser2448 (Santa Cruz, Cat. No. sc-293133) and their corresponding total proteins, Raf (Abcam, Cat. No. ab137435), ERK (Santa Cruz, Cat. No. sc-292838), Akt (Santa Cruz, Cat. No. sc-8312), mTOR (Santa Cruz, Cat. No. sc-1549), Icmt (Merck Inc. Cat. No. 09-119) and β-actin (Santa Cruz, Cat. No. sc-130656) were used.

2.4. Small interfering RNA (siRNA) and plasmid transfection

Transfections of siRNA or plasmid were performed with Lipofectamine TM 2000 and OptiMEM (Invitrogen, US) as per the manufacturer's protocol. ICMT siRNA and control SCR siRNA were purchased from Santa Cruz, US. The sequences of ICMT siRNA are the same as described previously [19]. Control plasmid (pDonor-255, Addgene, US) and plasmid containing human HRAS Q61L was a gift from Dominic Esposito (Addgene plasmid # 83186). Cells were processed for lysates preparation or cellular assays at 48 h post-transfection.

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