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Original Article

Blockade of Stearoyl-CoA-desaturase 1 activity reverts resistance to cisplatin in lung cancer stem cells

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ABSTRACT

Poor prognosis in lung cancer has been attributed to the presence of lung cancer stem cells (CSCs) which resist chemotherapy and cause disease recurrence. Hence, the strong need to identify mechanisms of chemoresistance and to develop new combination therapies. We have previously shown that Stearoyl-CoA-desaturase 1 (SCD1), the enzyme responsible for the conversion of saturated to monounsaturated fatty acids is upregulated in 3D lung cancer spheroids and is an upstream activator of key proliferation pathways β-catenin and YAP/TAZ. Here we first show that SCD1 expression, either alone or in combination with a variety of CSCs markers, correlates with poor prognosis in adenocarcinoma (ADC) of the lung. Treatment of lung ADC cell cultures with cisplatin enhances the formation of larger 3D tumor spheroids and upregulates CSCs markers. In contrast, co-treatment with cisplatin and the SCD1 inhibitor MF-438 reverts upregulation of CSCs markers, strongly synergizes in the inhibition of 3D spheroids formation and induces CSCs apoptosis. Mechanistically, SCD1 inhibition activates endoplasmic reticulum stress response and enhances autophagy. These data all together support the use of combination therapy with SCD1 inhibitors to achieve better control of lung cancer.

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Abbreviations: CSCs, Cancer Stem Cells; PE, Pleural Effusion; SFE, Sphere Forming Efficiency; NSCLC, Non Small Cell Lung Cancer; ADC, Adenocarcinoma; SCC, Squamous Cell Carcinomas; LCC, Large Cell Lung Cancer; DFS, Disease Free Survival; CDDP, Cisplatin; MF-438, (2-methyl-5-(6-(4-(2-(trifluoromethyl)phenoxy)piperidin-1-yl)pyridazin-3-yl)-1,3,4-hiadiazole); PD-L1, Programmed Death-Ligand 1; SCD1, Stearoyl-CoA Desaturase 1; SFAs, Saturated Fatty Acids; MUFAs, Monounsaturated Fatty Acids; DEAB, Diethylaminobenzaldehyde; BAAA, BODIPY-Aminoacetaldehyde; γH2AX, Nuclear Histone γH2AX; ALDH1A1, Aldehyde Dehydrogenase 1 family, member A1; CHOP (or DDIT3), DNA Damage Inducible Transcript 3; cPARP, Cleaved PARP.

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Introduction

Lung cancer is the most common cause of cancer-related deaths in the developed world [1–4]. In spite of the development of new therapeutic strategies the outcome of patients with lung cancer has only subtly improved over the past few decades, and the overall 5-year survival rate has remained very low (10–15%) [1,5,6]. Adenocarcinoma (ADC) is the most common histological type comprising of approximately 60% of non-small cell lung cancers (NSCLC) [2,3].

Although immunotherapy with checkpoint inhibitors has recently been approved for the treatment of patients which overexpress PD-L1 [7,8], platinum-based chemotherapy represents the standard first-line treatment for unselected patients with advanced NSCLC and second-line therapy in PD-L1 overexpressing patients that fail to respond to immunotherapy. A substantial proportion of patients have an unfavorable outcome due to the development of chemotherapy resistance and to recurrent disease thus indicating that chemotherapy is unable to eradicate residual cancer cells [9–11]. In this framework the identification of molecular targets that are overexpressed in chemotherapy-resistant cancer cells and are responsible for their survival is of utmost importance to developing new strategies that are capable of enhancing drug sensitivity and at prolonging survival.

According to the CSCs theory, tumorigenesis and cancer progression are due to a subset of phenotypically distinct cells characterized by unlimited self-renewal and enhanced clonogenic potential [12–16]. The eradication of the CSCs fraction is a challenging issue. It has been reported that lung CSCs are associated with higher recurrence rates [17]. In agreement with this, lung cancer with stem cell signatures has been associated with resistance to several anticancer drugs such as cisplatin, gemcitabine, docetaxel and with disease relapse [18–21].

Previous studies from our laboratory have highlighted the involvement of Stearoyl-CoA desaturase 1 (SCD1) in the survival of lung CSCs [22–24]. SCD1 is an iron-containing enzyme belonging to the family of fatty acids desaturases and represents a critical enzyme of lipid synthesis which catalyzes the conversion of saturated fatty acids (SFAs), into monounsaturated fatty acids (MUFAs). Our previous studies have shown that lung CSCs isolated from malignant pleural effusions are enriched for the expression of SCD1, and that this correlated with increased ALDH1A1 activity [22–26]. Moreover, SCD1 inhibition significantly suppressed the ability to form 3D spheroids, induced the selective apoptosis of ALDH1A1 positive cells and impaired tumor growth in vivo [23].

Even though a growing number of studies have demonstrated that SCD1 plays a key role in the development and maintenance of malignancy in several tumor types such as colon, ovary, thyroid, renal carcinomas, and more recently breast cancer [27–33], no investigations have been carried out to identify the prognostic and diagnostic relevance of SCD1 expression in combination with markers linked to stemness in patients affected by lung adenocarcinoma. Furthermore, the potential synergy between platinum therapy and SCD1 inhibition in lung adenocarcinoma has not yet been addressed.

In this paper, through a combination of analyses of gene expression databases, immunohistochemistry of human tumor samples and cell cultures of primary and established lung adenocarcinoma cell lines, we demonstrate that SCD1 is a diagnostic and prognostic marker able to predict the outcome for patients with lung ADC and a promising target for therapeutic intervention in combination with chemotherapy.

Materials and methods

Reagents

MF-438 was kindly provided by Ziga Jakopin. Cisplatin (CDDP) was purchased by Sigma, St. Louis, MO, USA.

Cell cultures

The NSCLC cell line, NCI-H460, was obtained from American Type Culture Collection (ATCC). PE2/15, PE4/15, PE5/15 and PEO/11 primary cultures were isolated from PE of ADC patients as previously described [22,23,25,26]. The study was approved by Ethics Committee (3382/25/09/2014). Cell cultures were maintained in RPMI-1640 (Sigma, St. Louis, MO, USA) supplemented with 10% FBS (Sigma, St. Louis, MO, USA) at 37 °C in a humidified atmosphere of 5% CO_2 in air. To maintain the integrity of collections, all the primary cell lines were maintained in culture no more than passages 6–10th. All cells were routinely checked for mycoplasma contamination and analyzed for morphology.

Sphere formation, MTT assay and drug treatment

Sphere propagation and MTT assays were performed as previously described [22,23,34] (see details in Supplementary materials and methods). For the determination of IC50, 1500 cells/well were suspended in sphere-forming medium and plated into an ultra-low adherent plate (Costar, USA) [22,23,34] in presence of a dilution series of 3-fold increments of CDDP or MF-438 (0.007–50 μM), alone or in a simultaneous or serial combination.

Evaluation of Sphere-Forming Efficiency (SFE) was determined by dividing the number of 3D spheres formed by the number of seeded cells on day 7, or 14 as specified. The quotient was then multiplied by 100.

For other experiments cells were cultured in the presence or absence of CDDP (0.5 μM) or MF-438 (1 μM) for 48 h, and harvested to perform ALDH1A1 activity, Real Time-PCR (RT-PCR), Western Blotting (WB) and FACS analyses.

siRNA transfection

We transfected small interfering RNA-targeting SCD-1 (Sigma) or control siRNA-A (sc-37007; Santa Cruz, CA, USA) into adherent cells using Lipofectamine RNAi MAX Reagents (Invitrogen), as previously described [23].

FACS analyses

Cell cycle distribution of cells was analyzed measuring cellular DNA content by flow cytometry. Spheroids were collected and fixed with 70% (v/v) ethanol. After 48 h cells were incubated with RNAse (10 μg/ml) and propidium iodide (10 μg/ml) for 30 min at 37 °C.

FACS-based Aldefluor assay (Stem Cell Technologies, Vancouver, BC, Canada) was carried out to identify the cells expressing ALDH1A1 activity according to Pisanu et al. [34]. Briefly, 3D spheroids (0.5–1.0 × 10^6) were incubated with ALDH1A1 substrate BODIPY-aminoacetaldehyde and/or with diethylaminobenzaldehyde (DEAB) (as a negative control (CTRL)) for 30 min. The same staining procedure was applied before sorting the cells with FACSAria (BD Biosciences). All data were acquired using an EPICS Coulter XL (Beckman-Coulter Inc.).

RT-PCR analyses

For RT-PCR experiments RNA was isolated and reverse-transcribed into cDNA as previously described [23] (see details and sequences of primers in Supplementary materials and methods).

WB analyses

For WB assays, cells were lysated as previously described [23]. Membranes were blotted with anti-GAPDH, anti-cPARP, anti-LC3I/LC3II (Sigma, St. Louis, MO, USA), anti-CHOP (Cell Signaling Technology, Beverly, MA, USA) primary antibodies and normalized over GAPDH and expressed as a fold-change relative to CTRL.

Immunofluorescence analyses and optical microscopy

For immunofluorescence (IF) analyses cells were fixed with 4% paraformaldehyde (PFA), permeabilized in 0.1% Triton-X100 (Sigma-Aldrich), and stained with anti-γH_2AX, anti-CHOP (Cell Signaling Technology, Beverly, MA, USA) antibody (or PBS alone as a negative CTRL). Immunofluorescence and morphology images were captured using an inverted microscope (Nikon, Tokyo, Japan), an Axiocam Camera (Zeiss) and analyzed using ZEN core software (Zeiss, Gottingen, Germany).

Immunohistochemistry

Archival human samples from the Istituto Nazionale Tumori “Fondazione Pascale” Institutional Biobank (47 adenocarcinomas (ADC), 32 squamous-cell carcinomas (SCC) and 10 healthy) (Table S1) obtained with informed and signed consent form, were stained with anti-SCD1 (clone CD.E10). SCD1 expression was scored by

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