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Telomerase-Mediated Strategy for Overcoming Non–Small Cell Lung Cancer Targeted Therapy and Chemotherapy Resistance

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Abstract

Standard and targeted cancer therapies for late-stage cancer patients almost universally fail due to tumor heterogeneity/plasticity and intrinsic or acquired drug resistance. We used the telomerase substrate nucleoside precursor, 6-thio-2′-deoxyguanosine (6-thio-dG), to target telomerase-expressing non–small cell lung cancer cells resistant to EGFR-inhibitors and commonly used chemotherapy combinations. Colony formation assays, human xenografts as well as syngeneic and genetically engineered immune competent mouse models of lung cancer were used to test the effect of 6-thio-dG on targeted therapy– and chemotherapy-resistant lung cancer human cells and mouse models. We observed that erlotinib-, paclitaxel/carboplatin-, and gemcitabine/cisplatin-resistant cells were highly sensitive to 6-thio-dG in cell culture and in mouse models. 6-thio-dG, with a known mechanism of action, is a potential novel therapeutic approach to prolong disease control of therapy-resistant lung cancer patients with minimal toxicities.

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Introduction

Lung cancer is the most common cause of cancer-related deaths [1]. However, tumor acquired drug resistance is one of the major reasons why chemotherapy and targeted therapies fail to provide durable responses [2, 3]. Almost universally, tumors develop resistance due to intratumor heterogeneity and/or different mechanisms such as target gene alterations (i.e., amplification of epidermal growth factor

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receptor [EGFR] and EGFR T790M mutation), downstream bypass signaling pathway activation (i.e., MET amplification or BRAF mutations), and phenotypic alterations (epithelial to mesenchymal transition), thus limiting the success of targeted therapies in lung cancer [4,5]. Osimertinib (AZD9291) is an FDA-approved EGFR inhibitor which is used to overcome drug resistance in non–small cell lung cancer (NSCLC) with the EGFR T790M mutation. Despite the impressive results of this drug, acquired resistance still develops, and little is known about drug resistance mechanisms [6]. In addition, there are diverse erlotinib resistance mechanisms that can emerge in what is termed "persister" derived resistant clones that arise from a single cell [7], indicating the complexity of resistance mechanisms. Likewise, while subsets of lung cancer patients have durable responses to checkpoint inhibitors, in the majority of cases, resistance also develops [8]. Thus, for all types of lung cancer systemic treatment modalities, there remains an outstanding need to develop new approaches to treat resistant tumors including biomarkers predictive signatures of response to any new treatment modalities to prolong disease control.

Telomerase is an almost universal biomarker in advanced human cancers [9,10]. Telomerase inhibitors are a potentially important class of targeted therapies; however, long-duration treatments result in hematological toxicities that prevent their advancement in clinical use. For example, a lead telomerase oligonucleotide, imetelstat (IMT), has not progressed well in clinical trials due to a long lag period to observe clinical benefit and drug-related hematological toxicities [11,12]. When IMT therapy is temporarily stopped, tumor telomerase is immediately reactivated and tumor telomeres rapidly regrow [13]. Thus, finding alternative strategies to target telomerase positive cancer cells is an urgent need.

6-thio-2′-deoxyguanosine (6-thio-dG), a modified nucleoside, is preferentially incorporated into telomeres but only in telomerase-positive cells [14]. When an altered nucleotide, 6-thio-dG, is incorporated into the telomere sequence, it leads to rapid telomere uncapping, genomic instability, and cell death. Therefore, while 6-thio-dG rapidly kills the telomerase-positive cancer cells, it has minimal effects on telomerase-negative normal cells. Additionally, we found that 6-thio-dG induced no significant toxicity in mice (no weight loss; no changes in hematological, renal, or liver functions) [14,15]. This led us in the current study to test the effect of 6-thio-dG on lung cancers that are resistant to platin-doublet chemotherapy or EGFR tyrosine kinase inhibitor–targeted therapies. We find that cells resistant to first-line standard chemotherapies or EGFR-targeted therapies remain sensitive to 6-thio-dG treatment at pharmacological doses. Together, our observations suggest that 6-thio-dG may be an effective therapeutic approach to prolong disease control in therapy-resistant tumors.

Materials and Methods

Cell Lines

The NCI and HCC lung cancer lines used were obtained from the UT Southwestern Hamon Center repository. Except when noted, NSCLC cell lines were grown in a Medium X (DMEM:199, 4:1, Hyclone, Logan, UT) supplemented with 10% cosmic calf serum (Hyclone, Logan, UT) without antibiotics and incubated in a humidified atmosphere with 5% CO_2 at 37°C. NSCLC cell lines were authenticated using the Power-Plex 1.2 kit (Promega, Madison, WI) and confirmed to match the DNA fingerprint library maintained by ATCC and confirmed to be free of mycoplasma by e-Myco kit (Boca Scientific, Boca Raton, FL). Human bronchial epithelial cells (HBECs) were cultured in bronchial epithelial growth medium (Lonza, Allendale, NJ) with an antibiotic solution (penicillin G–streptomycin–amphotericin B) and incubated in low oxygen (2%-3%) at 37°C. PC9-derived erlotinib-sensitive (PC9-1) and erlotinib-resistant clones [persister-derived erlotinib-resistant clone (PERC)9, PERC10, PERC13, PERC16, PC9-ER] were generated as previously described [7]. These cells were grown in RPMI-1640 (Sigma, St Louis, MO) supplemented with 5% fetal bovine serum (Hyclone, Logan, UT) without antibiotics. H1299 parental cells were treated long term with increasing doses of paclitaxel+carboplatin combination (2:3 ratio) to generate H1299 paclitaxel/carboplatin-resistant cells. Surviving cells were cultured in drug-free media until they repopulated the plate (one cycle); then a second treatment cycle was started. After 18 repetitive cycles of treatment, H1299 T18 paclitaxel/carboplatin-resistant cells were generated [16].

Drug Preparation

For *in vitro* studies, 6-thio-dG (Metkinen Oy, Kuopio, Finland) was dissolved in DMSO/water (1:1) to prepare 10 mM stock solutions, which were kept frozen at −20°C. A 1 mM, final concentration stock was prepared for *in vitro* experiments and added in fresh medium at different concentrations. Erlotinib HCl (Selleckchem, Houston, TX) was dissolved in DMSO to prepare 10 mM stock solutions, which were kept frozen at −80°C. A 2.5 μM erlotinib final concentration was used for *in vitro* experiments. Osimertinib and paclitaxel (Selleckchem) were dissolved in DMSO, and carboplatin (Selleckchem) was dissolved in water to prepare 10 mM stock solutions, which were kept frozen at −80°C. A 1 mM final concentration stock was prepared for *in vitro* experiments and added in fresh medium at different concentrations.

For mouse *in vivo* studies, 6-thio-dG was prepared in 5% DMSO for intraperitoneal (i.p.) injection or 0.4% (hydroxypropyl)methyl cellulose (Sigma, Saint Louis, MO) for oral gavage. Erlotinib was prepared in 15% Captisol (β-cyclodextrin, sulfobutyl ethers, sodium salts) (Cydex Pharmaceuticals, Lawrence, KS) for oral gavage. Gemcitabine HCl and cisplatin (Selleckchem) were dissolved in 0.9% NaCl saline solution for i.p. injections.

Cell Viability Assay

For IC_{50} determinations, a panel of NSCLC cell lines was screened with 6-thio-dG with a four-fold dilution series in 8 different points in 96-well plates. Cells were plated 24 hours prior to addition of drug, incubated for 4 days, and assayed using MTS (CellTiter 96 Aqueous One Solution Cell Proliferation Assay) according to the manufacturer's instructions (Promega). Cell number per well ranged from 500 to 4000 cells per well inversely proportional to doubling times (typically 2000/well). Dose-response curves were generated and IC_{50} calculated using in-house software DIVISA. All samples were analyzed in triplicate, and standard deviations are from two to three independent experiments.

For determination of viable cells with cell counting, normal HBEC cells (10,000 cells/cm^2) were treated with 6-thio-dG (1, 3, 10 μM) every 3 days for 2 weeks. The cells were counted at the end of first week and replated for a second week. H1299 parental (800 cells/cm^2) and T18 (1600 cells/cm^2) cells were grown in a Medium X (DMEM: