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Checkpoint kinase 1 protein expression indicates sensitization to therapy by checkpoint kinase 1 inhibition in non–small cell lung cancer

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ABSTRACT

Background: When presenting with advanced stage disease, lung cancer patients have <5% 5-y survival. The overexpression of checkpoint kinase 1 (CHK1) is associated with poorer outcomes and may contribute to therapy resistance. Targeting CHK1 with small-molecule inhibitors in p53 mutant tumors might improve the effectiveness of chemotherapy and radiotherapy in non–small cell lung cancer (NSCLC).

Methods: We evaluated CHK1 messenger RNA and protein levels in multiple NSCLC cell lines. We assessed cell line sensitization to gemcitabine, pemetrexed, and radiotherapy by CHK1 inhibition with the small molecule AZD7762 using proliferation and clonogenic cell survival assays. We analyzed CHK1 signaling by Western blotting to confirm that AZD7762 inhibits CHK1.

Results: We selected two p53 mutant NSCLC cell lines with either high (H1299) or low (H1993) CHK1 levels for further analysis. We found that AZD7762 sensitized both cell lines to gemcitabine, pemetrexed, and radiotherapy. Chemosensitization levels were greater, however, for the higher CHK1 protein expressing cell line, H1299, when compared with H1993. Furthermore, analysis of the CHK1 signaling pathway showed that H1299 cells have an increased dependence on the CHK1 pathway in response to chemotherapy. There was no increased sensitization to radiation in H1299 *versus* H1993.

Conclusions: CHK1 inhibition by AZD7762 preferentially sensitizes high CHK1 expressing cells, H1299, to anti-metabolite chemotherapy as compared with low CHK1 expressing H1993 cells. Thus, CHK1 inhibitors may improve the efficacy of standard lung cancer therapies, especially for those subgroups of tumors harboring higher expression levels of CHK1 protein.

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1. Introduction

Lung cancer is one of the most commonly diagnosed malignancies in the United States with more than 220,000 new cases each year. Even with advances in chemotherapy and radiotherapy survival rates for patients with advanced stage disease remain largely unchanged [1]. The best chemotherapeutic agents have limited impact with median patient survival only 11–13 mo [2]. Non–small cell lung cancer (NSCLC) comprises 85% of all lung cancers, with three major subtypes: adenocarcinoma, squamous, and large cell carcinoma. These subtypes are extremely heterogeneous with regards to the specific genetic mutations, which drive tumor growth. There are number of factors that contribute to limited chemotherapeutic efficacy, including cellular drug transporters, dose-related toxicities [3] and increased DNA repair mechanisms [4]. Because new oncogenic pathways with novel targets have been identified [5], emerging targeted therapies are an attractive strategy for improving survival in NSCLC. Epidermal growth factor receptor (EGFR) has proven to be an important therapeutic target as EGFR-mutated tumors are an identifiable subgroup of NSCLC's that may benefit from EGFR-targeted therapeutics. Unfortunately, in the Western population EGFR mutations occur in only 5%–10% of NSCLCs. Other gene mutations identified include anaplastic lymphoma kinase (ALK) fusion genes, p53, and KRAS [6]. With the notable exception of ALK kinase inhibitors in ALK fusion-positive lung adenocarcinomas, most therapies directed at these alterations have not shown favorable responses [7,8].

In recent years, targeting checkpoint kinase 1 (CHK1), an integral component of the DNA-damage response, with small molecule inhibitors has been proposed as one new approach for targeted therapy. A number of CHK1 inhibitors have been developed and have even begun to be used in clinical trials for various cancer types [9,10]. In response to DNA damage induced by cancer treatments, tumor cells activate a complex signaling network to arrest the cell cycle and enable DNA repair [11,12]. Critical molecules in the DNA damage response are p53 and the protein kinases, CHK1 and CHK2. CHK1 has been shown to contribute to therapy resistance and overall cell survival by inducing G_2 arrest and activating homologous recombinant repair [13]. It has been shown that inhibition of CHK1 increases chemotherapy and radiotherapy sensitivity in multiple tumor models, including lung [14–16]. Furthermore, sensitization by CHK1 inhibition appears to be tumor cell-selective and preferential in p53 mutant tumor types [17–19]. p53 has been shown to be an important factor in predicting CHK1 inhibitor-mediated sensitization, as p53 wild-type tumors are less sensitized to DNA damage in response to CHK1 inhibition.

We designed a study to assess the effects of the CHK1 inhibitor AZD7762 on NSCLC cell proliferation and clonogenic survival. Prior data from our laboratory [20] have been further analyzed and have suggested that increased CHK1 expression in primary human lung tumors is associated with poor survival. We also found that NSCLC cells have varying levels of CHK1 messenger RNA (mRNA) and protein expression. We hypothesized that the level of CHK1 expression would determine the ability of CHK1 inhibitors to sensitize tumor cells to chemotherapy and ionizing radiation. We sought to determine if AZD7762 in combination with radiation or the antimetabolite chemotherapies, pemetrexed, or gemcitabine could selectively target lung cancers that overexpress CHK1.

2. Methods

2.1. Cell culture, drugs, and reagents

H1993, H23, H1437 (adenocarcinomas) and H1299, and H460 (large cell carcinomas) cells were obtained from American Type Culture Collection (Manassas, VA) and grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS). H460-DNp53 cells were obtained from AstraZeneca [21]. Pemetrexed, and gemcitabine (Eli Lilly Company, Indianapolis, Indiana) were dissolved in phosphate buffered saline as a stock solution at 1 mM. AZD7762 (AstraZeneca) was dissolved in dimethyl sulfoxide as a stock solution at 10 mM. SMARTpool *CHK1* or non-targeting–control pool small interfering RNAs were purchased from Dharmacon (Lafayette, CO) and used according to the manufacturer's protocol.

2.2. Quantitative real-time polymerase chain reaction

RNA was isolated from H1993, H23, H1437, and H1299 cell lines by homogenizing cells in QIAzol reagent (Qiagen, Valencia, CA) and purifying RNA using RNeasy Mini Kits (Qiagen). Two microgram of total RNA was reverse transcribed using a High Capacity complementary DNA Transcription Kit (Applied Biosystems, Foster City, CA). *CHK1* transcripts were quantified by quantitative real-time polymerase chain reaction (qRT-PCR) using Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, Grand Island, NJ) in a Rotor-Gene 3000 thermocycler (Corbett Life Science, Valencia, CA). Relative expression levels were normalized to β-actin expression using the $2^{-\Delta\Delta}$ computed tomography method [22].

Primer sequences were as follows: ACTB (forward): 5′-\ATGTGGCCGAGGACTTTGATT-3′; ACTB (reverse): 5′- AGT GGGGTGGCTTTTAGGATG-3′ [23]; *CHK1* (forward): 5′–CGGT GGAGTCATGGCAGTGCCC-3′; *CHK1* (reverse): 5′- TCTGGACA GTCTACGGCACGCTTCA-3′.

2.3. Cell line microarray construction

Formalin-fixed, paraffin-embedded blocks of 48 cell lines were arrayed into a cell line microarray using the methodology of *Nocito et al.* [24]. Each cell line was represented by two 1 mm diameter cores.

2.4. Immunohistochemistry

Immunohistochemical staining was performed on the Dako Autostainer (Dako, Carpinteria, CA) using Dako EnVision + polymerized horseradish peroxidase and diaminobenzadine as the chromogen. Sections of deparaffinized cell line microarray were labeled overnight with CHK1 (rabbit monoclonal antibody, clone EP691Y, 1:100; Abcam, Cambridge, MA).