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CGK733 does not inhibit ATM or ATR kinase activity in H460 human lung cancer cells

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Dear Editor,

The purpose of this letter is to address the activity of CGK733, a small molecule that was reported to inhibit both ATM and ATR kinase activities and block checkpoint signaling with great selectivity [1]. This manuscript was retracted [2]. Nevertheless, CGK733 is marketed as an ATM and ATR kinase inhibitor and several manuscripts have reported that CGK733 has effects on human cells [3–7]. We have investigated the effects of CGK733 in cells exposed to either ionizing radiation (IR) or ultraviolet radiation (UV).

To determine whether CGK733 inhibits ATM kinase activity we examined the well-characterized IR-inducible ATM kinase-dependent phosphorylations on ATM serine 1981 [8] and CHK2 threonine 68 [9]. We treated H460 human lung cancer cells with 10 μM CGK733, 10 μM KU55933 or 1 μM KU60019 and exposed these to 5 Gy γ-rays from a ^{137}Cs source. KU55933 and KU60019 are inhibitors of ATM kinase activity [10,11]. We selected H460 because we previously documented the ATM-dependent effects of both KU55933 and KU60019 in these cells [12,13] and we selected a concentration of 10 μM CGK733 because this is the highest dose reported to inhibit the kinase activities of ATM and ATR in human cells.

We observed that 10 μM CGK733 did not inhibit the IR-induced phosphorylation of either ATM serine 1981 or CHK2 threonine 68 one hour following irradiation whereas both 10 μM KU55933 and 1 μM KU60019, which served as positive controls for the experiment, inhibited the IR-induced phosphorylation of both ATM serine 1981 and CHK2 threonine 68 (Fig. 1A). Similar results were obtained using CGK733 purchased from Tocris Bioscience and Sigma–Aldrich.

To determine whether CGK733 inhibits ATR kinase activity we examined the phosphorylation of ATM on serine 1981 and the well-characterized UV-induced

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phosphorylation on CHK1 serine 317 [14]. We treated H460 cells with 10 μM CGK733 or 10 μM ETP-46464 and exposed these to 10 J/m^2 UV. ETP-46464 is an inhibitor of ATR kinase activity [15]. We observed that 10 μM CGK733 did not inhibit the UV-induced phosphorylation of CHK1 serine 317 six hours following irradiation whereas 10 μM ETP-46464, which served as a positive control for the experiment, inhibited the UV-induced phosphorylation of CHK1 serine 317 (Fig. 1B). Further, the phosphorylation of ATM serine 1981 was increased in cells incubated in 10 μM ETP-46464 and exposed to 10 J/m^2 UV. This has been observed previously [15] and is consistent with a model in which inhibition of the kinase activity of ATR causes an accumulation of double-strand DNA breaks, perhaps as a result of the disruption of protein complexes that protect stalled replication from endonucleases.

We conclude that CGK733 does not inhibit ATM or ATR kinase activity in H460 human lung cancer cells.

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References

[1]. Won J, Kim M, Kim N, Ahn JH, Lee WG, Kim SS, Chang KY, Yi YW, Kim TK. Small molecule-based reversible reprogramming of cellular lifespan. Nat. Chem. Biol. 2006; 2:369–374. [PubMed: 16767085]

[2]. Won J, Kim M, Kim N, Ahn JH, Lee WG, Kim SS, Chang KY, Yi YW. Retraction: small molecule-based reversible reprogramming of cellular lifespan. Nat. Chem. Biol. 2008; 4:431. [PubMed: 18560433]

[3]. Cruet-Hennequart S, Glynn MT, Murillo LS, Coyne S, Carty MP. Enhanced DNA-PK-mediated RPA2 hyperphosphorylation in DNA polymerase etadeficient human cells treated with cisplatin and oxaliplatin. DNA Repair (Amst.). 2008; 7:582–596. [PubMed: 18289945]

[4]. Shrivastav M, Miller CA, De Haro LP, Durant ST, Chen BP, Chen DJ, Nick-oloff JA. DNA-PKcs and ATM co-regulate DNA double-strand break repair. DNA Repair (Amst.). 2009; 8:920–929. [PubMed: 19535303]

[5]. Alao JP, Sunnerhagen P. The ATM and ATR inhibitors CGK733 and caffeine suppress cyclin D1 levels and inhibit cell proliferation. Radiat. Oncol. 2009; 4:51. [PubMed: 19903334]

[6]. Takahashi A, Mori E, Su X, Nakagawa Y, Okamoto N, Uemura H, Kondo N, Noda T, Toki A, Ejima Y, Chen DJ, Ohnishi K, Ohnishi T. ATM is the predominant kinase involved in the phosphorylation of histone H2AX after heating. J. Radiat. Res. (Tokyo). 2010; 51:417–422. [PubMed: 20448412]

[7]. Cuffe S, Dowling CM, Claffey J, Pampillón C, Hogan M, Fitzpatrick JM, Carty MP, Tacke M, Watson RW. Effects of titanocene dichloride derivatives on prostate cancer cells, specifically DNA damage-induced apoptosis. Prostate. 2011; 71:111–124. [PubMed: 20665530]

[8]. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature. 2003; 421:499–506. [PubMed: 12556884]

[9]. Matsuoka S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science. 1998; 282:1893–1897. [PubMed: 9836640]

[10]. Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NM, Orr AI, Reaper PM, Jackson SP, Curtin NJ, Smith GC. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Res. 2004; 64:9152–9159. [PubMed: 15604286]

[11]. Golding SE, Rosenberg E, Valerie N, Hussaini I, Frigerio M, Cockcroft XF, Chong WY, Hummersone M, Rigoreau L, Menear KA, O'Connor MJ, Povirk LF, van Meter T, Valerie K.

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Improved ATM kinase inhibitor KU-60019 radiosensitizes glioma cells, compromises insulin, AKT and ERK prosurvival signaling, and inhibits migration and invasion. Mol. Cancer Ther. 2009; 8:2894–2902. [PubMed: 19808981]

[12]. White JS, Choi S, Bakkenist CJ. Irreversible chromosome damage accumulates rapidly in the absence of ATM kinase activity. Cell Cycle. 2008; 7:277–1284. [PubMed: 18235223]

[13]. White JS, Choi S, Bakkenist CJ. Transient ATM kinase inhibition disrupts DNA damage-induced sister chromatid exchange. Sci. Signal. 2010; 3:ra44. [PubMed: 20516478]

[14]. Zhao H, Piwnica-Worms H. ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. Mol. Cell. Biol. 2001; 21:4129–4139. [PubMed: 11390642]

[15]. Toledo LI, Murga M, Zur R, Soria R, Rodriguez A, Martinez S, Oyarzabal J, Pastor J, Bischoff JR, Fernandez-Capetillo O. A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. Nat. Struct. Mol. Biol. 2011; 18:721–727. [PubMed: 21552262]

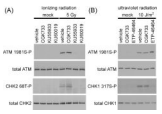

Fig. 1.

(A) Immunoblots of IR-inducible ATM kinase-dependent phosphorylations on ATM serine 1981 and CHK2 threonine 68. H460 human lung cancer cells were treated with 10 μM CGK733 (Tocris Bioscience), 10 μM KU55933 or 1 μM KU60019 (KuDOS Pharmaceuticals) from 30 min prior to exposure to 5 Gy γ-rays until harvest at 60 min following the insult. Briefly, whole cell extracts were prepared in lysis buffer: 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 50 mM NaF, 1% Tween-20, 0.5% NP40 and 1× protease inhibitor mixture (Roche Applied Science, Indianapolis, IN). Cleared cell extracts were resolved in 3–8% Tris–acetate gels (Invitrogen, Carlsbad, CA) and immunoblotted with rabbit monoclonal anti-ATM 1981S-P antisera (EP1890Y, Epitomics), generic mouse monoclonal anti-ATM antisera (MAT3-4G10/8, Sigma), rabbit polyclonal anti-CHK2 68T-P (2661, Cell Signaling) and mouse monoclonal anti-CHK2 (1C12, Cell Signaling). (B) Immunoblots of the phosphorylation of ATM on serine 1981 and ultraviolet radiation (UV)-induced phosphorylation on CHK1 serine 317. H460 human lung cancer cells were treated with either 10 μM CGK733 or 10 μM ETP-46464 from 30 min prior to exposure to 10 J/m^2 UV radiation until harvest at 6 h following the insult. Cleared cell lysates were immunoblotted with rabbit polyclonal anti-CHK1 317S-P (2344, Cell Signaling) and mouse monoclonal anti-CHK1 (2G1D5, Cell Signaling).

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