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mTORC1 and DNA-PKcs as novel molecular determinants of sensitivity to Chk1 inhibition

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ABSTRACT

Background: Chk1 inhibitors are currently under clinical evaluation as single agents and in combination with cytotoxic chemotherapy. Understanding determinants of sensitivity and novel combinations is critical for further clinical development.

Methods: Potentiation of mTOR inhibitor cytotoxicity by the Chk1 inhibitor V158411 was determined in p53 mutant colon cancer cells. DNA damage response, expression levels of repair proteins, cell cycle effects and the contribution of alternative DSB repair pathways were further evaluated by western blotting and high content analysis.

Results: mTOR inhibitors AZD8055, RAD-001, rapamycin and BEZ235 induced synergistic cytotoxicity with the Chk1 inhibitor V158411 in p53 mutant colon cancer cells. Reduced FANCD2, RAD51 and RPA70, core proteins in homologous recombination repair (HRR) and interstrand crosslink repair (ICLR), following inhibition of mTOR was associated with increased V158411 induced DSBs and caspase 3-independent cell death. Dual mTOR and Chk1 inhibition activated DNA-PKcs. Cells defective in DNA-PKcs exhibited increased resistance to V158411 with Chk1 expression closely correlated to DNA-PKcs expression in various types of cancer.

Conclusions: Down regulation of proteins involved in HRR or ICLR by mTOR inhibitors is associated with increased sensitivity of human tumours to Chk1 inhibitors such as V158411. High levels of DNA-PKcs may be a potential biomarker to stratify patients to Chk1 inhibitor therapy alone or in combination with mTOR inhibitors.

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1. Introduction

The DNA damage response (DDR) is the result of adaptation to the high level of DNA damage sustained by the genome from endogenous and environmental sources on a daily basis. Activation of the DDR results in a number of cellular responses including checkpoint activation and cell cycle arrest, initiation of DNA repair, regulation of transcription and apoptosis (Bucher and Britten, 2008; Dai and Grant, 2010; Liu et al., 2000; Smith et al., 2010). The checkpoint kinase Chk1 is a central, key component of the DDR and is activated by phosphorylation on serine 317 and serine 345 by the ATR and ATM kinases (Niida et al., 2007; Tapia-Alveal et al., 2009) and auto-phosphorylation on serine 296 (Ng et al., 2004). Activation is in response to DNA damage in the form of single-stranded regions of DNA and double-strand breaks (DSB)

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induced by both endogenous (e.g. DNA replication stress) or exogenous (e.g. genotoxic agents or radiation) sources. Chk1 activation results in the inhibition/degradation of the Cdc25 family of phosphatases. Cell cycle progression is therefore prevented through the maintenance of inhibitory phosphorylation (Y15/T14) on Cdk1 and Cdk2. Biochemical and genetic studies have demonstrated Chk1 to be essential and indispensable for the S- and G2/M checkpoints (Cho et al., 2005; Liu et al., 2000). The G1/S checkpoint is dysfunctional in most human cancers, e.g. by p53 mutation or functional inactivation (Massague, 2004), rendering cancer cells reliant on Chk1/Chk2 for checkpoint activation in the presence of endogenous or exogenous DNA damage.

With the aim of selectively exploiting tumour-specific G1 checkpoint dysfunction, Chk1 inhibitors have been developed (reviewed in (Chen et al., 2012; Garrett and Collins, 2011)). The pre-clinical and clinical development of these inhibitors has focussed on their ability to potentiate the cytotoxicity of genotoxic chemotherapy drugs (such as gemcitabine, irinotecan or cisplatin) or ionising radiation. All of these agents induce DNA damage and activate the DDR resulting in cell cycle arrest. Inhibition of Chk1 following genotoxic stress induced by these agents results in checkpoint abrogation, inhibition of DNA repair and induction of cell death in cells particularly in those with a defective p53 response. This approach is currently being evaluated in the clinic in a range of Phase I and II trials.

In addition to its role in the DDR, Chk1 has been demonstrated to be important for replication origin firing (Ge and Blow, 2010; Maya-Mendoza et al., 2007; Petermann et al., 2010), high rates of replication fork progression and replication fork stabilization (Smith-Roe et al., 2013; Syljuasen et al., 2005). This evidence implicating a role for Chk1 in the cell-cycle and DNA replication in the absence of exogenous DNA damage suggests combining a Chk1 inhibitor with alternative, molecularly targeted therapeutic agents may be a rational therapeutic option. Synergism with Chk1 inhibitors (UCN-01, LY2603618 and PF-477736 and the pan Chk1/Chk2 inhibitor (AZD7762) has so far been observed with MEK inhibitors in glioblastoma (Tang et al., 2012a) and cytokinetically quiescent multiple myeloma (Pei et al., 2011); with PARP inhibitors in breast cancer (Mitchell et al., 2010; Shibata et al., 2011; Tang et al., 2012a); with Src family kinase inhibitors in glioblastoma (Tang et al., 2012b), multiple myeloma (Dai et al., 2008, 2011) and breast cancer (Mitchell et al., 2011); with farnesyl transferase inhibitors in leukaemia and myeloma (Dai et al., 2005); and with the mTOR inhibitor rapamycin in leukaemia (Hahn et al., 2005). Chk1 inhibitors have demonstrated single agent activity in cancers harbouring defects in DNA repair pathways or with high levels of replicative stress including neuroblastoma (Cole et al., 2011), melanoma (Brooks et al., 2013), leukaemia and lymphoma (Bryant et al., 2014b; Ferrao et al., 2012; Murga et al., 2011), breast cancer (Bryant et al., 2014a; Shibata et al., 2011), and cell lines defective in components of the Fanconi's anaemia DNA repair pathway (Chen et al., 2009).

V158411 is a potent, cell selective inhibitor of Chk1 that potentiates the cytotoxicity of a range of DNA damaging cancer therapeutic agents, such as gemcitabine and camptothecin, in p53 mutant cancer cell lines (Rawlinson and Massey, 2014). We report here for the first time the remarkable synergy between V158411 and a variety of mTOR inhibitors in the cytotoxicity to models of human solid tumours and the surprising observation that high expression of DNA-PKcs, a key component of the DNA DSB repair pathway, non-homologous end-joining (NHEJ), confers sensitivity to V158411.

2. Materials and methods

2.1. Cell lines and cell culture

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HT29, SW620 and Colo205 cell lines were purchased from the American Type Culture Collection (ATCC), established as a low passage cell bank and then routinely passaged in our laboratory for less than 3 months after resuscitation. These were routinely cultured in DMEM containing 10% FCS and 1% penicillin/streptomycin at 37 °C in a normal humidified atmosphere supplemented with 5% CO_2. AA8, V3 and xrs-6 cells were obtained from Keith Caldecott at the Genome stability unit at University of Sussex. M059J, DNA-PKcs-deficient human glioblastoma cells (Anderson et al., 2001), were grown in DMEM supplemented with 10% FBS. M059J-Fus-1 (M059J transfected with a portion of chromosome 8 carrying the DNA-PKcs gene; (Virsik-Kopp et al., 2004)) cells were cultured in media with 400 μg/ml G418. Cells were authenticated by STR profiling (LGC Standards, Teddington UK).

2.2. Compounds

Solid stocks were purchased from the indicated suppliers and prepared as concentrated stock solutions in the appropriate solvent: gemcitabine (Apin Chemicals Inc), 20 mM in H_2O; BEZ235 (Selleckchem), 2 mM in DMSO; rapamycin (LC Laboratories), 5 mM in DMSO; AZD6244 (Selleckchem), 20 mM in DMSO; sorafenib (Selleckchem), 20 mM in DMSO; sunitinib (Selleckchem), 20 mM in DMSO; AZD8055 (Selleckchem), 5 mM in DMSO; RAD-001 (Selleckchem), 5 mM in DMSO; BEZ235 (Selleckchem), 2 mM in DMSO. V158411 was from Vernalis Research and prepared as a 20 mM DMSO stock. Compounds were serially diluted in DMSO to 500× or 1000× then to 5× or 10× in complete media before addition to cells to yield a 1× final concentration.

2.3. Potentiation assays

5000 cells per well were seeded in 96-well plates and incubated overnight. Cells were treated with a 10-point titration of cytotoxic chemotherapeutic agent in the presence of a fixed concentration of Chk1 inhibitor for 72 h. The effect on cell proliferation was determined using CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS, Promega) and read on a Victor plate reader (Perkin Elmer).

2.4. Anchorage independent growth assays

1500 cells/well in 0.4% low melting point agarose (SeaPlaque, Lonza) in complete media were plated on to 96-well plates coated with 0.8% low melting point agarose in complete media. Wells were subsequently overlaid with complete media

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