MOLECULAR ONCOLOGY XXX (2014) 1–15

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Mitotic arrest and slippage induced by pharmacological inhibition of Polo-like kinase 1

Monika Raab[a], Andrea Krämer[a], Stephanie Hehlgans[b], Mourad Sanhaji[a], Elisabeth Kurunci-Csacsko[a], Christina Dötsch[a], Gesine Bug[c], Oliver Ottmann[c], Sven Becker[a], Fiona Pachl[d], Bernhard Kuster[d,e], Klaus Strebhardt[a,e,*]

[a]Department of Gynecology, School of Medicine, Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany
[b]Department of Radiotherapy and Oncology, Goethe University, Germany
[c]Department of Medicine, Hematology/Oncology, Goethe University, Germany
[d]Technische Universität München, Emil Erlenmeyer Forum 5, 85354 Freising, Germany
[e]German Cancer Consortium (DKTK), Heidelberg, Germany

ARTICLE INFO

Article history:
Received 8 July 2014
Accepted 25 July 2014
Available online ■

Keywords:
Polo-like kinase 1
Cell cycle
Mitotic arrest
Slippage

ABSTRACT

Exposure to drugs that interfere with microtubule dynamics block cell cycle progression at mitosis by prolonged activation of the spindle assembly checkpoint (SAC). Cells can evade mitotic arrest and proceed to interphase without chromosome segregation by a process termed mitotic slippage that involves Cyclin B1 degradation without checkpoint inactivation. Here, we explored the cellular response to small-molecule inhibitors of Polo-like kinase 1 (Plk1), an important regulator of cell division. We found that the clinical Plk1 inhibitors BI 2536 and BI 6727, both unexpectedly, induced a dose-dependent cellular drug response: While mitotic arrest was induced in cancer cell lines and primary non-transformed cells across the entire range of concentrations tested, only high concentrations seemed to promote mitotic slippage. Since this observation contrasts with the effects expected from studies reporting RNAi-mediated Plk1 depletion in cancer cells, we wondered whether both ATP-competitive inhibitors target unknown kinases that are involved in signaling from the spindle assembly checkpoint (SAC) and might contribute to the mitotic slippage. A chemical proteomics approach used to profile the selectivity of both inhibitors revealed that SAC kinases are not targeted directly. Still, the activities of Cdk1/Cyclin B1 and Aurora B, which plays important roles in the error correction of false microtubule-kinetochore attachments and in checkpoint signaling, were shown to be downregulated at high inhibitor concentrations. Our data suggest that the inhibition of Plk1 activity below a certain threshold influences Aurora B activity via reduced phosphorylation of Fox M1 and Survivin leading to diminished levels of Aurora B protein and alteration of its subcellular localization. Within the spectrum of SAC proteins that are degraded during mitotic slippage, the degradation of Cyclin B1 and the downregulation of Aurora B activity by Plk1 inhibition seem to be critical promoters of mitotic slippage. The results

* *Corresponding author*. Department of Obstetrics and Gynecology, J.W. Goethe-University, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany. Tel.: +49 69 6301 6894; fax: +49 69 6301 6364.
E-mail address: strebhardt@em.uni-frankfurt.de (K. Strebhardt).
http://dx.doi.org/10.1016/j.molonc.2014.07.020

Please cite this article in press as: Raab, M., et al., Mitotic arrest and slippage induced by pharmacological inhibition of Polo-like kinase 1, Molecular Oncology (2014), http://dx.doi.org/10.1016/j.molonc.2014.07.020

indicate that careful dose-finding studies in cancer trials are necessary to limit or even prevent mitotic slippage, which could be associated with improved cancer cell survival.

1. Introduction

The polo-like family of serine/threonine kinases plays a crucial role in the regulation of cell cycle progression (Archambault and Glover, 2009; Barr et al., 2004; van de Weerdt and Medema, 2006). Because cancer cells require Plk1 for survival (Liu and Erikson, 2003; Spankuch-Schmitt et al., 2002b), Plk1 has been investigated intensively as a target for novel anti-cancer agents (Liu et al., 2011; McInnes et al., 2006; Strebhardt, 2010). Potent small-molecule inhibitors of mammalian Plk1, BI 2536 and BI 6727, which inhibit Plk1 enzyme activity at low nanomolar concentrations, cause mitotic arrest, induce apoptosis in human cancer cell lines of different origin and are currently evaluated in multiple clinical cancer trials (Lenart et al., 2007; Rudolph et al., 2009). Antimitotic agents like Plk1 inhibitors or compounds that interfere with microtubule dynamics, including taxanes and vinca alkaloids induce the spindle assembly checkpoint (SAC) that prevents the progression to anaphase until all the chromosomes are properly attached to kinetochores and are under the right tension (Manchado et al., 2012; Musacchio and Salmon, 2007). The anaphase-promoting complex/cyclosome (APC/C) is an E3-ubiquitin ligase that triggers mitotic exit during normal mitotic progression mostly by targeting Cyclin B1 for degradation, thus inhibiting Cdk1 activity. The SAC targets CDC20, a co-factor of the APC/C. In particular, by keeping CDC20 in check the APC/C remains inactive which prevents the destruction of two key substrates, Cyclin B1 and Securin. However, long-term activation of the SAC during exposure to antimitotic agents may cause the levels of proteins essential to maintain mitotic arrest to fall, triggering mitotic slippage, which occurs when cells exit mitosis, without chromosome segregation or cell division (Andreassen et al., 1996; Brito and Rieder, 2006). Spontaneous mitotic slippage takes place through slow ubiquitylation of Cyclin B1 by APC/C^{Cdc20} and subsequent proteasome-dependent degradation despite mitotic checkpoint activity. The core components of the SAC include MAD2, BUBR1/Mad3, BUB3, MAD1 and the kinases BUB1, multipolar spindle -1 (MPS1) and Aurora B. Moreover, Aurora B is part of the chromosomal passenger complex (CPC), which comprises INCENP, Borealin, Survivin and Aurora B (Musacchio and Salmon, 2007). The CPC, which can be found at chromosomes and kinetochores in early mitosis, controls the attachment of microtubules to chromosomes, sister chromatid cohesion and the SAC. The use of the Aurora B-specific inhibitors, like Basertib, stimulates mitotic slippage in different types of cancer cells (Marxer et al., 2013; Yang et al., 2007).

Comprehensive investigations of cell populations and different cell types have revealed that cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs encompassing death during mitosis, mitotic slippage and death in the subsequent interphases, mitotic slippage and cell survival, or no mitotic entry (Gascoigne and Taylor, 2008; Marxer et al., 2013; Sakurikar et al., 2012). The determination of the type of cellular response to be expected upon treatment with a specific antimitotic agent and the study of factors that determine the outcome is important for the identification of predictive factors that determine the patients' response and thus for a tailored and rational design of clinical trials testing efficacy of novel anticancer agents.

In this study we examined the dose-dependent response of cancer cells and primary human cells to the clinical Plk1 inhibitors BI 2536 and BI 6727. While in the entire range of concentrations tested both inhibitors induced mitotic arrest, at higher concentrations mitotic slippage was observed. Our study revealed that activities of Cdk1/Cyclin B1 and Aurora B are inhibited at higher drug concentrations accompanied by reduced protein levels and dyslocalized Aurora B suggesting that reduced levels of Aurora B activity and Cyclin B1 contribute to mitotic slippage of human cells upon exposure to Plk1 inhibitors.

2. Materials and methods

2.1. Preparation of primary cells and cell culture

HUVEC and fibroblasts were isolated and cultured as described (Raab et al., 2011) Keratinocytes were grown in Dermalife® K Medium Complete Kit (Cell Systems). Immortalized human retinal pigment epithelial cells (hTERT-RPE1) expressing Plk1 ^{wt} or Plk1 ^{as} were propagated as described (Burkard et al., 2012). Cancer lines were purchased from the American Type Culture Collection, cultured with 10% FCS (PAA) at 37 °C with 5% CO_2 in a humidified atmosphere: MEM (Sigma) for HeLa, RPMI 1640 (PAA) for HL-60. MDA-MB-468 cells were grown in Leibovitz's L-15 (PAA) without CO_2. For Plk1 inhibition, cells were treated with 10–2000 nM BI 2536 or BI 6727 (ICS).

Bone marrow samples were obtained from AML patients at diagnosis or according to study schedule. All patients gave written informed consent and collection of samples was approved by the ethics committee of the Goethe-University. Cells were collected with heparinized syringes and purified on a Ficoll density gradient. For long-term cultures (LTC)-AML (FFM5, FFM12), mononuclear cells were isolated as described (Rossmanith et al., 2001). AML cells were maintained in X-Vivo 10 (Lonza)/10% FCS HyClone (Perbio)/1% L-glutamine (Sigma) and cytokines hIL-3 (20 ng/ml), hTPO

Please cite this article in press as: Raab, M., et al., Mitotic arrest and slippage induced by pharmacological inhibition of Polo-like kinase 1, Molecular Oncology (2014), http://dx.doi.org/10.1016/j.molonc.2014.07.020