

Research article

## SWITCHING p53-DEPENDENT GROWTH ARREST TO APOPTOSIS VIA THE INHIBITION OF DNA DAMAGE-ACTIVATED KINASES

PAVLA HUBLAROVA<sup>1</sup>, KRISTINA GREPLOVA<sup>2</sup>, JITKA HOLCAKOVA<sup>1</sup>,  
 BORIVOJ VOJTESEK<sup>1</sup> and ROMAN HRSTKA<sup>1\*</sup>

<sup>1</sup>Department of Oncological and Experimental Pathology, <sup>2</sup>Department of Laboratory Medicine, Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 53, Brno, Czech Republic

**Abstract:** Cisplatin and doxorubicin are widely used anticancer drugs that cause DNA damage, which activates the ATM-Chk2-p53 pathway in cancer cells. This activation leads to cell cycle block or apoptosis, depending on the nature of the DNA damage. In an attempt to enhance the effects of these agents, we inhibited ATM/ATR and Chk2, which are known upstream regulators of p53. The cancer cell lines A2780 and ARN8, bearing the wild-type p53 protein, were used to study changes in p53 activation and trans-activation. Our results suggest that the G<sub>1</sub>-checkpoint, normally activated by DNA damage, is functionally overcome by the action of kinase inhibitors that sensitize cells to apoptosis. Both inhibitors show these effects, albeit with variable intensity in different cell lines, which is promising for other studies and theoretically for use in clinical practice.

**Key words:** Protein p53, ATM/ATR kinases, Chk2, Inhibitors of DNA damage-activated kinases, Doxorubicin, Cisplatin

### INTRODUCTION

The *TP53* tumour suppressor gene encodes a 393-amino acid transcription factor that plays a crucial role in determining the cellular response to various stress conditions [1, 2]. The level of p53 protein is mainly regulated at the post-translational level by the MDM2 protein, while expression of MDM2 is

---

\* Author for correspondence. e-mail: [hrstka@mou.cz](mailto:hrstka@mou.cz), tel.: +420543133306, fax: +420543211169

Abbreviations used: cisPt – cisplatin, dox – doxorubicin, DSB – double-strand break, inh 1 – CGK733 inhibitor ATM/ATR, inh 2 – Chk2 inhibitor II, qRT-PCR – quantitative real-time polymerase chain reaction

activated by p53 at the transcription level, forming a negative feedback loop to maintain p53 protein at low levels under normal conditions [3, 4].

DNA damage was the first type of stress found to activate p53. Based on this, p53 has been widely regarded as “the guardian of the genome” [5]. The activation of p53 in response to DNA damage occurs through multiple signalling pathways, which include cascades of Ser/Thr kinases, especially ATM, ATR, Chk1 and Chk2 [6, 7]. However, while ATM responds to DNA double-strand breaks induced by ionizing radiation and other radiomimetic drugs, ATR mainly detects single-strand breaks arising from stalled replication forks or in response to ultraviolet radiation. In addition, ATM takes part in multiple cell cycle phase checkpoints, whereas ATR is primarily involved in the S-phase checkpoint. The downstream phosphorylation targets of ATM and ATR are the effector Ser/Thr kinases Chk1 and Chk2, which in turn phosphorylate partially overlapping residues in other target proteins to induce cell cycle arrest and facilitate DNA repair. Whereas Chk1 is activated by ATR phosphorylation on Ser317 and Ser345, Chk2 is activated by ATM phosphorylation on Thr68 (reviewed in [8]). ATM plays a key role in sensing DNA damage and in propagating signals that modulate protective cellular responses to genotoxic agents [9]. Functional ATM is required for optimal p53 induction and activation following cellular exposure to agents that induce DNA double-strand breaks (DSB) [10, 11]. Recent findings suggest a direct role for ATM in the DNA damage-induced phosphorylation of p53 on Ser15 [12, 13] and an indirect role in Ser20 phosphorylation via control of the activation of Chk2 [14]. In part, these phosphorylation events are believed to block the abilities of MDM2 both to inhibit p53 transcriptional activity and to target p53 for degradation. As such, the level of active p53 protein rises, leading to enhanced expression of key p53 target genes that function as cell cycle inhibitors and inducers of apoptosis.

DNA-damaging agents such as doxorubicin and cisplatin can cause cell cycle arrest as well as apoptosis. Which of these occurs is largely dependent on p53. It was confirmed that this induction is dependent on ATM/ATR, the kinase known to activate p53 in response to DNA damage [15]. We investigated how direct modulation of ATM/ATR and Chk2 signalling pathways can influence wild-type p53 protein accumulation, post-translational modifications and transcriptional activity. Specifically, we used two kinase inhibitors: ATM/ATR Kinase Inhibitor CGK733 and Chk2 Inhibitor II. We analysed their effects during combined treatment with cisplatin or doxorubicin.

## **MATERIAL AND METHODS**

### **Cell lines and treatments**

In this study, we used two cancer cell lines expressing the wild-type p53 protein: ARN8 (melanoma) and A2780 (ovarian carcinoma). The cell lines were respectively cultivated in DMEM or RPMI 1640, supplemented with 10% foetal bovine serum and with L-glutamine (300 mg/l; all from Gibco Life Technologies

Corp., Carlsbad, CA, USA). The cultivation was done at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>.

The cells were treated with the DNA-damaging cytostatic agents 10 µM cisplatin (Cisplatin, Teva, Petach Tikva, Israel) and 0.5 µM doxorubicin (Adriablastica, Ebewe, Unterach, Austria). The kinases included in the DNA damage signalling were blocked by 5 µM ATM/ATR Kinase Inhibitor CGK733 or by 10 µM CHK2 Inhibitor II (2-(4-(4-chlorophenoxy)phenyl)-1H-benzimidazole-5-carboxamide; both from Calbiochem, Merck, Poole, UK). All agents were applied alone and in combinations for 16 h on cells in 50% confluency.

### SDS-PAGE and immunoblotting

This method was carried out as described previously [16] with some differences. 20 µg of whole protein per well was loaded into 10% SDS-PAGE gels. The proteins in the gels were transferred onto nitrocellulose membranes in a Bio-Rad Trans-Blot SD semi-dry transfer cell applying 200 mA, or in wet mode in Mini-PROTEAN cells with a mini-blotting module applying 100 V for 2 h in the transfer buffer (all Bio-Rad Laboratories, Hercules, CA, USA). The membranes were probed overnight with the monoclonal antibodies specific anti-p53 (DO-1) [17], anti-p53-S392 (FP392) [18], anti-MDM2 (Ab2A9) [19], anti-p53-S15 (Cell Signaling Technology, Inc., Danvers, USA), anti-actin AC-40 (Sigma-Aldrich Inc., St. Louis, MO, USA), anti-p21<sup>WAF-1</sup> (Ab-1) and anti-PARP-1 (Ab2; both Calbiochem). The performed immunodetection of anti-actin AC-40 confirmed the equal protein loading.

### Quantitative real-time PCR (qRT-PCR)

Total cellular RNA was extracted with an RNeasy Mini kit (Qiagen, Hilden, Germany) according to the protocol for cultivated cells. cDNA synthesis was carried out with 500 ng of total RNA by using M-MLV reverse transcriptase and random hexamer primers (both Fermentas Life Sciences, Burlington, Ontario, Canada) in a total volume of 34 µl according to the manufacturer's protocol. Triplicate samples were subjected to qRT-PCR analysis using the SYBR Green or Taq Man systems in an ABI PRISM 7000. The relative quantitation of gene expression was determined via the comparative CT method. As an endogenous control, we used 18S rRNA (all from Applied Biosystems, Foster City, CA, USA). The primer pairs used for each gene were:

p21 <sup>WAF1</sup>	forward (F)	5'CTGGAGACTCTCAGGGTCGAA3',
	reverse (R)	5'AAGATGTAGAGCGGCCTTTG3';
MDM2	F	5'TTTGAAGTTATTAAGACTGTTGGTG3',
	R	5'ACAATATGTTGTTGCTTCTCATCA3';
GADD45	F	5'TTTTGCTGCGAGAACGACAT3',
	R	5'GACTTTCCCGGCAAAAACAA3';
NOXA	F	5'CTGTCCGAGGTGCTCCAGTT3',
	R	5'TCCTGAGTTGAGTAGCACAC3';
PUMA	F	5'CCAAACGTGACCACTAGCCT3',
	R	5'ACAGGATTCACAGTCTGGGC3'.

### **Cell cycle analysis by flow cytometry**

Trypsinized treated cells were washed twice in PBS, and resuspended in 500  $\mu$ l of 50 mg/l propidium iodide solution for 2 h at room temperature. The cell cycle analysis was performed using a flow cytometer FC500 (Beckman Coulter, Krefeld, Germany) and the propidium iodide fluorescence emission signal was detected in the FL3 channel. The percentages of cells in each phase of the cell cycle were determined using MultiCycle-AV software (Phoenix Flow Systems, San Diego, CA, USA). The quantification of apoptosis was performed via analyses of sub  $G_0$  DNA content, as described previously [20, 21]. Events that fell within the hypodiploid region (sub  $G_0$ ) were accounted for as apoptotic events.

### **Detection of apoptosis using the Annexin V assay**

The treated cells were trypsinized and adjusted to  $1 \times 10^6$  cells/ml in a culture medium. The annexin V-biotin apoptosis detection assay (Calbiochem) was performed according to manufacturer's protocol. The data was analysed using a Cell chip in Agilent 2100 bioanalyzer running Apoptosis series II software, all according to manufacturer's protocol (all Agilent technologies, Waldbronn, Germany).

## **RESULTS**

### **Inhibition of the ATM/ATR pathway down-regulates p53 activity**

Both cisplatin and doxorubicin are widely used chemotherapeutic agents that cause DNA damage. In response to these drugs, the cellular levels of p53 are increased and the protein itself is post-translationally stabilized and activated as a transcriptional factor. To analyze the role of ATM and Chk2 in p53 regulation in response to doxorubicin and cisplatin, we used specific inhibitors for these kinases. Importantly, neither of the inhibitors showed a cytotoxic effect at the used concentrations nor did they affect level of p53 protein when used alone.

Cells of the human ovarian carcinoma line A2780 were simultaneously treated with each inhibitor and with doxorubicin or cisplatin, and they showed a significant decrease in the level of p53 Ser15 and Ser392 phosphorylation. There was also a significant decrease in p53 transcriptional activity, as shown by the down-regulation of the *p21<sup>WAF1</sup>* and *MDM2* genes (Fig. 1A). The effect of the two inhibitors on p53 activity in response to DNA damage was also confirmed at the mRNA level via qRT-PCR analysis. We determined the mRNA levels of *p21<sup>WAF1</sup>*, *MDM2*, *GADD45*, *PUMA* and *NOXA* to comprise all the most important p53 regulated pathways. The decreasing activity of p53 seen at the protein level led to decreased promoter activation of these genes (Fig. 1B). These results clearly confirm the decrease in p53 trans-activation capability caused by combined treatment with the inhibitors and doxorubicin or cisplatin, compared to the effect of these drugs alone.



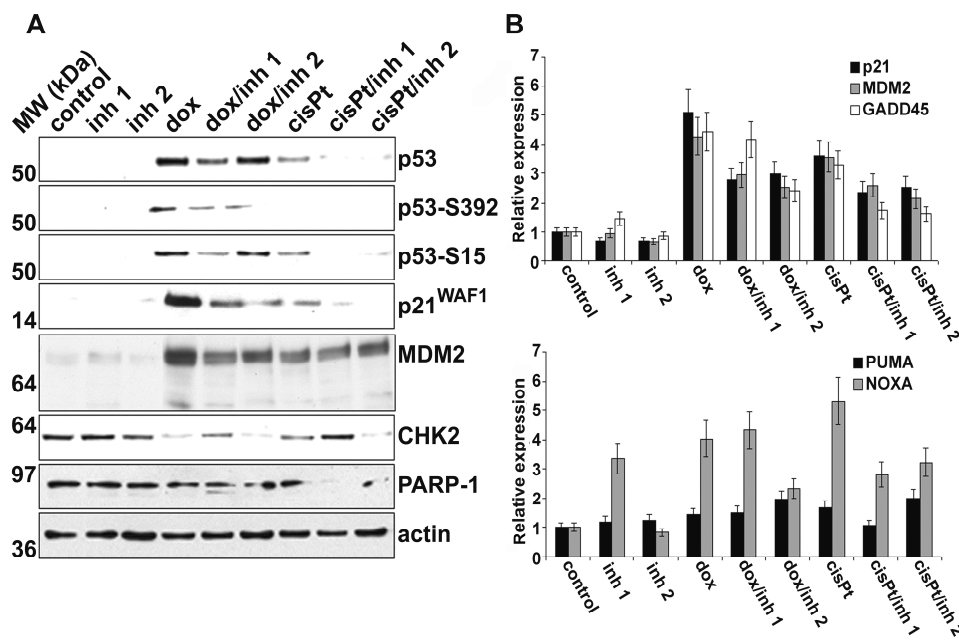


Fig. 1. An analysis of the cellular response to DNA-damaging agents and/or selected inhibitors in A2780 cells. A – Determination of the p53 cellular level, p53 Ser15, Ser392 phosphorylation and p53 transactivation activity via immunoblotting analysis. B – qRT-PCR analysis of several p53 down-regulated genes. Inh 1 – inhibitor ATM/ATR, inh 2 – Chk2 inhibitor II, dox – doxorubicin, cisPt – cisplatin.

To generalize these findings, we performed the same analysis with another cancer cell line expressing wild-type p53. We chose the ARN8 cell line, which is derived from a malignant melanoma. As with the A2780 cells, we observed a clear decrease in the level of p53 Ser15 and Ser392 phosphorylation followed by a loss of p53 transcriptional activity, validated at both the RNA and protein levels (Fig. 2). The overall effect of the two inhibitors leads to down-regulation of the p53 downstream cascade in both cell lines. The found differences in gene expression levels in A2780 cells derived from ovarian carcinoma compared to ARN8 cells derived from melanoma could be attributed to their different origin.

### The effect of combined treatment on the cell cycle and apoptosis

Flow cytometry analysis of propidium iodide-stained cells revealed that drug treatment reduces the number of cells blocked in the G<sub>1</sub> phase. Interestingly, the combination of inhibitor ATM/ATR and doxorubicin enhances the proportion of cells in the sub-G<sub>0</sub> phase, especially in ARN8 cells (Fig. 3). Quantifications of the sub-G<sub>0</sub> phase are shown in Tab. 1. Consistent with these findings, we found apoptotic cleavage of PARP-1 in both cell lines after application of the ATM/ATR inhibitor together with doxorubicin (Fig. 1A and 2A), indicating a potential new cancer treatment strategy based on the inhibition of ATM/ATR combined with doxorubicin treatment.

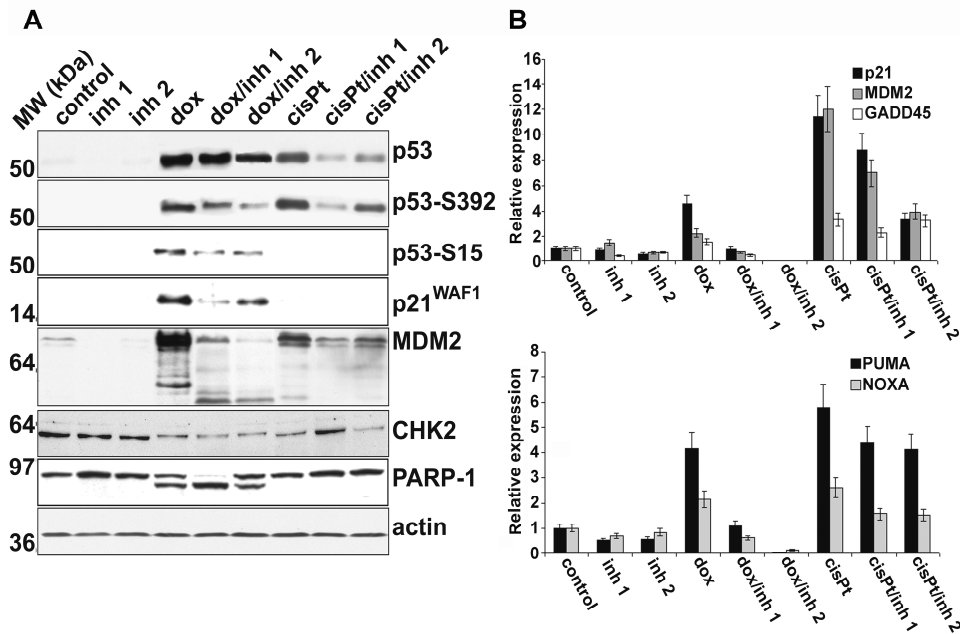


Fig. 2. An analysis of the cellular response to DNA-damaging agents and/or selected inhibitors in ARN8 cells. A – Determination of the p53 cellular level, the p53 Ser15 and Ser392 phosphorylation and the p53 transactivation activity via immunoblotting analysis. B – qRT-PCR analysis of several p53 down-regulated genes. Inh 1 – inhibitor ATM/ATR, inh 2 – Chk2 inhibitor II, dox – doxorubicin, cisPt – cisplatin.

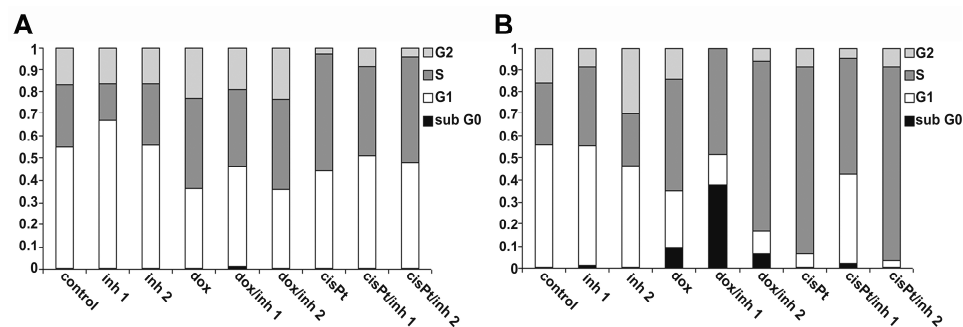


Fig. 3. A cell cycle analysis of A2780 (A) and ARN8 (B) cells via flow cytometry. Inh 1 – inhibitor of ATM/ATR, inh 2 – Chk2 inhibitor II, dox – doxorubicin, cisPt – cisplatin.

To support our results, we additionally analysed apoptosis at a very early stage using the annexin V method (Fig. 4). The annexin assay confirmed that combining the ATM/ATR inhibitor with doxorubicin enhances apoptosis in ARN8 cells while maintaining the level of apoptosis in A2780 cells. Interestingly, this assay revealed a significant effect of Chk2 inhibitor II in combination with both cytostatic agents in A2780 cells.

Tab. 1. The percentage of cells in the sub-G<sub>0</sub> phase revealed by flow cytometry analysis. The mean number of cells in the sub-G<sub>0</sub> phase was calculated from two measurements  $\pm$  standard deviation (SD). Inh 1 – inhibitor of ATM/ATR, inh 2 – Chk2 inhibitor II, dox – doxorubicin, cisPt – cisplatin.

Treatment	A2780	ARN8
control	0.225 $\pm$ 0.007	0.955 $\pm$ 0.018
inh 1	0.770 $\pm$ 0.078	1.639 $\pm$ 0.057
inh 2	0.272 $\pm$ 0.004	0.320 $\pm$ 0.042
dox	0.485 $\pm$ 0.049	9.801 $\pm$ 0.185
dox/inh 1	1.497 $\pm$ 0.134	38.147 $\pm$ 1.106
dox/inh 2	0.508 $\pm$ 0.013	6.485 $\pm$ 0.423
cisPt	0.942 $\pm$ 0.017	0.604 $\pm$ 0.016
cisPt/inh 1	0.784 $\pm$ 0.018	2.384 $\pm$ 0.096
cisPt/inh 2	0.650 $\pm$ 0.042	0.442 $\pm$ 0.010

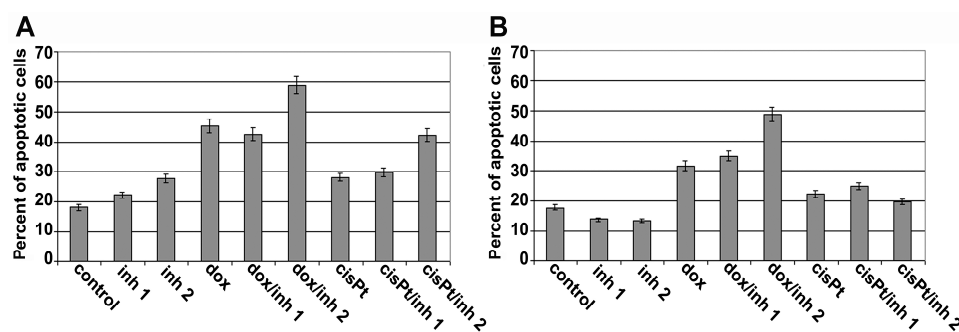


Fig. 4. An analysis of apoptosis in A2780 (A) and ARN8 (B) cells via the Annexin V assay. Inh 1 – inhibitor of ATM/ATR, inh 2 – Chk2 inhibitor II, dox – doxorubicin, cisPt – cisplatin.

## DISCUSSION

The outcome of DNA damage can be either DNA repair with cell survival or apoptotic cell death. Despite the extensive investigation of the DNA damage response, the molecular mechanism that determines whether the damage will be repaired or whether the damaged cells will undergo apoptosis is largely unknown. The lack of such knowledge significantly limits our understanding of cancer cell drug resistance and efficacious cancer treatment. Similarly, it is not entirely clear how the initial DNA lesions induced by different DNA-damaging agents are detected and lead to corresponding cellular signals, including p53 activation [22, 23].

Doxorubicin is a topoisomerase-II stabilizing agent that primarily causes DSBs, which activate ATM kinase. In addition, due to its structure, doxorubicin directly intercalates into DNA and generates reactive oxygen species, mainly

hydroxyl radicals, thereby contributing to the activation of ATM pathways [24]. Doxorubicin induces phosphorylation of six serine residues in p53 – 6, 9, 15, 20, 46 and 392 – in an ATM-dependent manner [24], where Ser15 plays a central role. Cisplatin induces DNA damage primarily at guanine residues, generating monoadducts, intrastrand crosslinks and interstrand crosslinks, which predominantly correlate with cisplatin cytotoxicity [25-28]. Cisplatin treatment induces p53 phosphorylation at Ser20 and at Ser15. This is due to its ability to activate ATR, which is the candidate kinase for phosphorylation of Ser15 by this drug [29].

Our results show decreased phosphorylation of p53 at Ser15 after exposure to combined treatment with inhibitors and DNA-damaging agents, which implicates lower p53 trans-activation activity identified by measuring the expression of the *p21<sup>WAF1</sup>* and *MDM2* genes at both the mRNA and protein levels. This result is also supported by the determination of the expression of the *PUMA*, *NOXA* and *GADD45* genes involved in the p53-driven apoptotic pathway.

The phosphorylations of p53 at Ser15 by the ATM and ATR kinases [13, 30-32] and Ser20 by human Chk2 kinase [33] after DNA damage play critical roles in p53 stabilization by interfering with MDM2 binding [7, 34, 35]. Cells lacking functional ATM fail to elicit p53/p21<sup>WAF1</sup> activation in response to DNA damage, as this pathway is kinetically delayed and quantitatively reduced, which results in the disruption of G<sub>1</sub> checkpoint arrest [36]. p21<sup>WAF1</sup> can serve as a dual regulator of cellular responses and its regulation seems to be critical for the cellular outcome following DNA damage [37]. p21<sup>WAF1</sup> is a critical target protein in the response to DNA-damaging agents [38-41], and it is induced by p53-dependent and p53-independent pathways in DNA-damaged cells. It was postulated that an imbalance of cell cycle signals or failure to arrest the cell cycle may trigger the apoptotic programme [42]. p21<sup>WAF1</sup> has an inhibitory effect on doxorubicin-induced apoptosis through the inhibition of caspase-3, but pre-activation of p21<sup>WAF1</sup> prior to doxorubicin treatment enhances apoptosis [42]. In our study, we decided to block the p53-p21<sup>WAF1</sup> pathway by inhibiting the upstream DNA damage-activated kinases ATM/ATR and Chk2, which resulted in clear down-regulation of p21<sup>WAF1</sup> at both the mRNA and protein levels and consequently, a functional overcoming of G<sub>1</sub> checkpoint arrest. We can speculate that during the acceleration or disruption of the G<sub>1</sub> checkpoint, cancer cells can accrue other genome changes, which further increases instability and could be accumulated through cell division and then can lead to cell death. Our cell cycle analysis indicates that inhibitor ATM/ATR in combination with cytostatic agents causes shortening of the S phase in the two cell lines compared to cells treated with either doxorubicin or cisplatin alone. This data supports the fact that inhibiting ATM/ATR and their downstream signalling pathways including p53 results in increased apoptosis and eventually in the abrogation of the G<sub>2</sub> blockage [43]. Accordingly, Chk2 inhibitor II was shown as highly specific without specific impact on other effector kinases activated by active

ATM or ATR such as Chk1, which also can partially induce DNA damage repair (reviewed in [8]). Using this approach would be beneficial for the treatment of tumours with wild-type p53, which can delay the G<sub>0</sub>/G<sub>1</sub> transition for DNA damage repair. For example, in the therapy of breast cancer, the activation of ATM by cytostatic agents is undesirable due to side effects [24], thus the potential use of inhibitors blocking ATM-dependent pathways can contribute to more effective treatment protocols and an improved long-term outcome of therapy.

We conclude that the specific inactivation of the ATM/ATR-dependent DNA damage response profoundly affects the sensitivity of cancer cells to DNA-damaging agents. Importantly, both of the tested inhibitors showed non-toxic effects at the used concentrations, but they effectively inhibited the functions of ATM/ATR and CHK2 in response to DNA damage. In consequence, their action functionally decreases the phosphorylation of p53 and down-regulates the induction of p53 downstream proteins, so cell cycle arrest and DNA repair are disrupted, and the survival of the cancer cells is threatened by p53-independent apoptosis or mitotic catastrophe. We believe that all these attributes of inhibitors look promising for the elimination of cancer cells in clinical practice.

**Acknowledgments.** This study was supported by the following grants: GACR 301/08/1468 and P301/10/1615, IGA MZCR NS/9812-4 and MZ0MOU2005. We are grateful to Dr. P. J. Coates for his critical reading of the paper, and to Ms. T. Kolarova and Ms. S. Babcanova for their technical assistance.

## REFERENCES

1. Ko, L.J. and Prives, C. p53: puzzle and paradigm. **Genes Dev.** 10 (1996) 1054-1072.
2. Levine, A.J. p53, the cellular gatekeeper for growth and division. **Cell** 88 (1997) 323-331.
3. Barak, Y., Juven, T., Haffner, R. and Oren, M. mdm2 expression is induced by wild type p53 activity. **EMBO J.** 12 (1993) 461-468.
4. Wu, X., Bayle, J.H., Olson, D. and Levine, A.J. The p53-mdm-2 autoregulatory feedback loop. **Genes Dev.** 7 (1993) 1126-1132.
5. Lane, D.P. Cancer. p53, guardian of the genome. **Nature** 358 (1992) 15-16.
6. Saito, S., Goodarzi, A.A., Higashimoto, Y., Noda, Y., Lees-Miller, S.P., Appella, E. and Anderson, C.W. ATM mediates phosphorylation at multiple p53 sites, including Ser(46), in response to ionizing radiation. **J. Biol. Chem.** 277 (2002) 12491-12494.
7. Siliciano, J.D., Canman, C.E., Taya, Y., Sakaguchi, K., Appella, E. and Kastan, M.B. DNA damage induces phosphorylation of the amino terminus of p53. **Genes Dev.** 11 (1997) 3471-3481.
8. Bartek, J. and Lukas, J. Chk1 and Chk2 kinases in checkpoint control and cancer. **Cancer Cell.** 3 (2003) 421-429.

9. Fedier, A., Schlamming, M., Schwarz, V.A., Haller, U., Howell, S.B. and Fink, D. Loss of atm sensitises p53-deficient cells to topoisomerase poisons and antimetabolites. **Ann. Oncol.** 14 (2003) 938-945.
10. Shiloh, Y. The ATM-mediated DNA-damage response: taking shape. **Trends Biochem Sci.** 31 (2006) 402-410.
11. Canman, C.E., Wolff, A.C., Chen, C.Y., Fornace, A.J., Jr. and Kastan, M.B. The p53-dependent G1 cell cycle checkpoint pathway and ataxia-telangiectasia. **Cancer Res.** 54 (1994) 5054-5058.
12. Canman, C.E., Lim, D.S., Cimprich, K.A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M.B. and Siliciano, J.D. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. **Science** 281 (1998) 1677-1679.
13. Banin, S., Moyal, L., Shieh, S., Taya, Y., Anderson, C.W., Chessa, L., Smorodinsky, N.I., Prives, C., Reiss, Y., Shiloh, Y. and Ziv, Y. Enhanced phosphorylation of p53 by ATM in response to DNA damage. **Science** 281 (1998) 1674-1677.
14. Hirao, A., Kong, Y.Y., Matsuoka, S., Wakeham, A., Ruland, J., Yoshida, H., Liu, D., Elledge, S.J. and Mak, T.W. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. **Science** 287 (2000) 1824-1827.
15. Lin, W.C., Lin, F.T. and Nevins, J.R. Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. **Genes Dev.** 15 (2001) 1833-1844.
16. Muller, P., Ceskova, P. and Vojtesek, B. Hsp90 is essential for restoring cellular functions of temperature-sensitive p53 mutant protein but not for stabilization and activation of wild-type p53: implications for cancer therapy. **J. Biol. Chem.** 280 (2005) 6682-6691.
17. Vojtesek, B., Bartek, J., Midgley, C.A. and Lane, D.P. An immunochemical analysis of the human nuclear phosphoprotein p53. New monoclonal antibodies and epitope mapping using recombinant p53. **J. Immunol. Methods** 151 (1992) 237-244.
18. Blaydes, J.P. and Hupp, T.R. DNA damage triggers DRB-resistant phosphorylation of human p53 at the CK2 site. **Oncogene** 17 (1998) 1045-1052.
19. Chen, J., Marechal, V. and Levine, A.J. Mapping of the p53 and mdm-2 interaction domains. **Mol. Cell Biol.** 13 (1993) 4107-4114.
20. Sheard, M.A., Krammer, P.H. and Zaloudik, J. Fractionated gamma-irradiation renders tumour cells more responsive to apoptotic signals through CD95. **Br. J. Cancer** 80 (1999) 1689-1696.
21. Sheard, M.A., Vojtesek, B., Janakova, L., Kovarik, J. and Zaloudik, J. Up-regulation of Fas (CD95) in human p53 wild-type cancer cells treated with ionizing radiation. **Int. J. Cancer** 73 (1997) 757-762.
22. Wang, D. and Lippard, S.J. Cellular processing of platinum anticancer drugs. **Nat. Rev. Drug Discov.** 4 (2005) 307-320.

23. Siddik, Z.H. Cisplatin: mode of cytotoxic action and molecular basis of resistance. **Oncogene** 22 (2003) 7265-7279.
24. Kurz, E.U., Douglas, P. and Lees-Miller, S.P. Doxorubicin activates ATM-dependent phosphorylation of multiple downstream targets in part through the generation of reactive oxygen species. **J. Biol. Chem.** 279 (2004) 53272-53281.
25. McHugh, P.J., Spanswick, V.J. and Hartley, J.A. Repair of DNA interstrand crosslinks: molecular mechanisms and clinical relevance. **Lancet Oncol.** 2 (2001) 483-490.
26. Kartalou, M. and Essigmann, J.M. Recognition of cisplatin adducts by cellular proteins. **Mutat. Res.** 478 (2001) 1-21.
27. Chaney, S.G., Campbell, S.L., Bassett, E. and Wu, Y. Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. **Crit Rev. Oncol. Hematol.** 53 (2005) 3-11.
28. Dronkert, M.L. and Kanaar, R. Repair of DNA interstrand cross-links. **Mutat. Res.** 486 (2001) 217-247.
29. Damia, G., Filiberti, L., Vikhanskaya, F., Carrassa, L., Taya, Y., D'Incalci, M. and Broggin, M. Cisplatin and taxol induce different patterns of p53 phosphorylation. **Neoplasia** 3 (2001) 10-16.
30. Khanna, K.K., Keating, K.E., Kozlov, S., Scott, S., Gatei, M., Hobson, K., Taya, Y., Gabrielli, B., Chan, D., Lees-Miller, S.P. and Lavin, M.F. ATM associates with and phosphorylates p53: mapping the region of interaction. **Nat. Genet.** 20 (1998) 398-400.
31. Lakin, N.D., Hann, B.C. and Jackson, S.P. The ataxia-telangiectasia related protein ATR mediates DNA-dependent phosphorylation of p53. **Oncogene** 18 (1999) 3989-3995.
32. Tibbetts, R.S., Brumbaugh, K.M., Williams, J.M., Sarkaria, J.N., Cliby, W.A., Shieh, S.Y., Taya, Y., Prives, C. and Abraham, R.T. A role for ATR in the DNA damage-induced phosphorylation of p53. **Genes Dev.** 13 (1999) 152-157.
33. Chehab, N.H., Malikzay, A., Appel, M. and Halazonetis, T.D. Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. **Genes Dev.** 14 (2000) 278-288.
34. Chehab, N.H., Malikzay, A., Stavridi, E.S. and Halazonetis, T.D. Phosphorylation of Ser-20 mediates stabilization of human p53 in response to DNA damage. **Proc. Natl. Acad. Sci. U. S. A.** 96 (1999) 13777-13782.
35. Shieh, S.Y., Ikeda, M., Taya, Y. and Prives, C. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. **Cell** 91 (1997) 325-334.
36. Delia, D., Fontanella, E., Ferrario, C., Chessa, L. and Mizutani, S. DNA damage-induced cell-cycle phase regulation of p53 and p21waf1 in normal and ATM-defective cells. **Oncogene** 22 (2003) 7866-7869.
37. Hill, R., Bodzak, E., Blough, M.D. and Lee, P.W. p53 Binding to the p21 promoter is dependent on the nature of DNA damage. **Cell Cycle** 7 (2008) 2535-2543.

38. Noda, A., Ning, Y., Venable, S.F., Pereira-Smith, O.M. and Smith, J.R. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. **Exp. Cell Res.** 211 (1994) 90-98.
39. Lu, Y., Tatsuka, M., Takebe, H. and Yagi, T. Involvement of cyclin-dependent kinases in doxorubicin-induced apoptosis in human tumor cells. **Mol. Carcinog.** 29 (2000) 1-7.
40. Lu, Y., Yamagishi, N., Yagi, T. and Takebe, H. Mutated p21(WAF1/CIP1/SDI1) lacking CDK-inhibitory activity fails to prevent apoptosis in human colorectal carcinoma cells. **Oncogene** 16 (1998) 705-712.
41. Luo, Y., Hurwitz, J. and Massague, J. Cell-cycle inhibition by independent CDK and PCNA binding domains in p21Cip1. **Nature** 375 (1995) 159-161.
42. Tang, J.J., Shen, C. and Lu, Y.J. Requirement for pre-existing of p21 to prevent doxorubicin-induced apoptosis through inhibition of caspase-3 activation. **Mol. Cell Biochem.** 291 (2006) 139-144.
43. Crescenzi, E., Palumbo, G., de Boer, J. and Brady, H.J. Ataxia telangiectasia mutated and p21CIP1 modulate cell survival of drug-induced senescent tumor cells: implications for chemotherapy. **Clin. Cancer Res.** 14 (2008) 1877-1887.