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PI3K inhibitors changed the p53-induced response of Saos-2 cells from growth arrest to apoptosis

Shuo Ren,[a] ChongFeng Gao,[a,1] Lilin Zhang,[b,2] Katsuro Koike,[c] and Nobuo Tsuchida[a,*]

[a] *Department of Molecular Cellular Oncology and Microbiology, Graduate School of Medicine and Dentistry, Tokyo Medical Dental University, Bunkyo-ku, Tokyo 113-8549, Japan*
[b] *Department of Cellular Physiological Chemistry, Graduate School of Medicine and Dentistry, Tokyo Medical Dental University, Bunkyo-ku, Tokyo 113-8549, Japan*
[c] *Department of Gene Research, The Cancer Institute (JFCR), Toshimaku, Tokyo 170, Japan*

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Abstract

p53 is activated by stress leading to oncogenic alteration, which induces either cell cycle arrest or apoptosis, although the mechanism involved in this decision has not been fully clarified as yet. This work was undertaken to change the cellular response by inducing apoptosis with PI3K inhibitors to Saos-2 cells that had been growth-arrested in both G1 and G2/M by the wild-type activity of temperature-sensitive (*ts*) p53. We found that the PI3K/Akt inhibitors LY294002 and wortmannin, but not the MEK inhibitor U0126, were capable of inducing apoptosis in growth-arrested Saos-2 cells, as assessed by an increase in the sub-G1 population, pyknotic nuclei, and DNA ladder formation. We detected the cleavage of caspases 9 and 3, and PARP after LY294002 addition, accompanied by a loss of cytochrome *c* from the mitochondria, and observed Bax translocation to the mitochondria and down-regulation of phospho-Akt, suggesting that blocking of survival signals triggered the apoptotic signal through the mitochondrial apoptotic pathway. It is thus suggested that the PI3K/Akt pathway played an important role in determining cell fate between growth arrest and apoptosis.
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P53 acts as a tumor suppressor primarily by inducing either cell cycle arrest or apoptosis in response to cellular stress leading to oncogenic alteration [1,2]. These cellular responses are mediated largely through the function of p53 as a transcriptional activator or repressor, targeting a diverse range of genes. Among transcriptionally activated proteins, $p21^{waf1}$ is the most important protein involved in cell cycle arrest at both G1 and G2/M checkpoints. $P21^{waf1}$ inhibits cyclin D or E/CDK in G1 arrest and cyclin B/cdc2 for G2/M arrest [3,4]. On the contrary, transcriptionally activated pro-apoptotic proteins are classified into two groups: one works through the death-receptor pathway and the other through the mitochondrial apoptotic pathway. While a few proteins work in the death receptor pathway, more work in the latter pathway including Bax, Noxa, Puma, and p53AIP [5–8]. These proteins are involved in the release of cytochrome *c* from the mitochondria into the cytosol. Cytochrome *c* in the cytosol then activates caspase 9 in the apoptosome together with dATP and Apaf1, which in turn activate downstream execution-type caspases such as caspases 3, 6, and 7 [9,10]. These caspases cleave many cellular substrates, ultimately leading to cell death [11].

Although each of the growth arrest and apoptosis pathways has been well studied, it has been reported that additional pathways are involved in determining which of the two cellular responses is triggered upon p53 activation. Two pathways play important roles in cell survival [12–16], namely the phosphatidylinositol 3-OH kinase (PI3K)/Akt pathway and the mitogen-activated protein kinase (MAPK) pathway. PTEN, one of the

* Corresponding author. Fax: +81-3-5803-0189.
E-mail address: tsuchida.mcom@tmd.ac.jp (N. Tsuchida).
[1] Present address: Van Andel Research Institute, 333 Bostwick Ave. NE, Grand Rapids, MI 49503, USA.
[2] Present address: Department of Post-genomics and Diseases, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.

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p53-target proteins and a tumor suppressor protein, was reported to down-regulate the PI3K/Akt survival signal [17], while HB-EGF [18], also a p53-target protein, up-regulates ERK1/2 phosphorylation and PI3K/Akt signaling cascades, being favorable for cell survival.

Previously, we showed that overexpression of a temperature-sensitive (*ts*) p53 mutant (p53A138V) induced cell cycle arrest in both the G1 and G2/M phases in Saos-2 cells at the permissive temperature, although the same *ts* mutant expression in Jurkat cells induced apoptosis [19,20]. In this study, we attempted to modulate growth-arrested cells to induce apoptosis by applying PI3K and MEK inhibitors. We found that the PI3K/Akt inhibitors LY294002 and wortmannin, but not the MEK inhibitor U0126, were capable of changing p53-induced growth arrest to apoptosis.

Materials and methods

Chemicals and antibodies. The inhibitors used were LY294002 (Alexis Biochemicals, San Diego, CA) and wortmannin (Wako Chemicals, Tokyo, Japan) for PI3K and U0126 (New England Biolabs, Boston, MA) for MEK. Geneticin (G-418) was from Sigma Chemicals (St. Louis, MO).

The antibodies used for immunological studies were anti-Bax (N-20) and anti-$p21^{waf1}$ (C-19) (Santa Cruz Biotechnology, California), anti-caspase-3 and -9, anti-PARP, anti-ERK2, anti-phospho-ERK1/2, anti-AKT, and anti-phospho-Akt (Ser-473) (Cell Signaling Technology, Beverly, MA); Anti-COX IV was from Molecular Probes (Eugene, OR). Anti-cytochrome *c* was from BD Transduction Laboratories (Tokyo, Japan).

Cell culture conditions and drug treatment. S138V cells carrying cytomegalovirus (CMV) promoted human p53 138Val mutant and Sneo cells carrying the CMV vector with neomycin-resistant gene were established previously [20]. The cells were grown in Dulbecco's modified Eagle's medium (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum, gentamycin sulfate (10 μg/ml), and geneticin (G-418, 400 μg/ml). For the treatment with kinase inhibitors, 0.5 μM wortmannin, 10 μM LY294002, or 10 μM U0126 was added to the culture medium and incubated at 37.5 °C for 1 h before and during the temperature shift-down or after culturing cells at either 37.5 or 32.5 °C for 24 h. Control treatments were done with DMSO at the same concentrations used for the drug treatment.

Cytosolic release of cytochrome c from and translocation of Bax to the mitochondria. The analysis was performed according to the method of Gottlieb and Gransville [21]. Briefly, the cells were collected by scraping, washed with phosphate-buffered saline (PBS), and suspended in digitonin lysis buffer (75 mM KCl, 1 mM NaH_2PO_4, 8 mM Na_2HPO_4, 250 mM sucrose, and 190 μg/ml digitonin). After 5 min on ice the cells were spun down for 5 min at 12,000*g* at 4 °C with a microcentrifuge, and the supernatant and pellet fractions were assayed for cytochrome *c* and Bax levels by Western blot.

Protein extraction and Western blot analysis. The cells were harvested by scraping off from the culture dishes, collected by centrifugation, and re-suspended in RIPA buffer (20 mM Tris–HCl, pH 7.6, 5 mM EDTA, 150 mM NaCl, 0.5% Nonidet P-40, 50 mM NaF, 1 mM β-glycerophosphate, 1 mM $Na_3VO_4 \cdot 4H_2O$, and 5 mM $Na_4P_2O_7 \cdot 10H_2O$) containing a protease inhibitor cocktail, Complete (Roche Diagnostics, Tokyo, Japan). Protein concentrations were then measured using a DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). The cell extracts were mixed with 2× loading buffer, boiled for 5 min, and chilled on ice, centrifuged, subjected to 10–15% SDS–PAGE, and electrophoretically transferred to a PVDF membrane. Each membrane was incubated with primary antibodies as described previously [22]. Briefly, the membranes were washed with tris-buffered saline and incubated with secondary antibodies conjugated with peroxidase and the signal was detected by the enhanced chemiluminescence (ECL) system (Amersham, Tokyo, Japan).

DNA fragmentation analysis. DNAs for fragmentation analysis were prepared as previously described [22]. Briefly, the cells (2×10^6) were collected, washed with cold PBS, suspended in a 40 μl mixture of 0.2 M Na_2HPO_4:0.1 M citric acid (192:8 v/v), incubated at room temperature for 60 min, and then centrifuged at 2000*g* for 30 min. The supernatants were treated with 0.02% Nonidet P-40 and RNase A (1 μg/ml final concentration) at 37.5 °C for 30 min. Proteinase K (1 μg/ml) was added and the mixture was incubated at 50 °C for 30 min. Aliquots (10 μl) were loaded on a 1.5% agarose gel and electrophoresis was carried out at 5 V/cm for 1 h in 1× TBE (89 mM Tris-borate and 2 mM EDTA, pH 8.0) buffer. The DNA was stained with ethidium bromide (0.5 μg/ml) and visualized under a UV illuminator.

FACS analysis to measure the sub-G1 population. The cells were trypsinized, collected by centrifuging at 350*g* for 2 min, and then stained with propidium iodide using a Cycle Testing Plus DNA Reagent Kit (Becton–Dickinson, Mountain View, CA). DNA contents were analyzed by a FACS analyzer (FACScan, Becton–Dickinson). A total of 10,000 cells were counted per sample and the data were processed using the Cell Quest software (Becton–Dickinson).

Results

PI3K inhibitors modulated ts p53-induced cell cycle arrest to apoptosis in Saos-2 cells

When Saos-2 cells expressing *ts* p53 (S138V) were cultured at the permissive temperature (32.5 °C), the cell growth was arrested at G1 and G2/M, but not at the non-permissive temperature (37.5 °C) (Fig. 1A, top panel), as we reported previously [20]. Using this system, we attempted to change the cellular response of S138V cells from growth arrest to apoptosis by treating them with the PI3K inhibitors LY294002 (10 μM) or wortmannin (0.5 μM) 1 h before the temperature shift-down and during the low temperature incubation for 24 h. The treatment induced significant apoptosis at the permissive temperature when compared with that at high temperature, as assessed by the increase in the sub-G1 fraction (Fig. 1A, lower two panels). The increase in the sub-G1 population in Sneo cells harboring an empty vector, after the inhibitor treatment, was not significant when compared at both temperatures (data not shown).

The fragmentation analysis of DNAs prepared at the same time point also showed typical ladder formation in PI3K-inhibitor-treated S138V cells only at the permissive temperature (Fig. 1B). However, under the same conditions U0126 (10 μM) had little effect on inducing apoptosis, as the sub-G1 population and DNA fragmentation were not significantly detected (data not shown).

These results taken together suggested that the PI3K inhibitors but not the MEK inhibitor could change the cellular response of S138V cells from growth arrest to

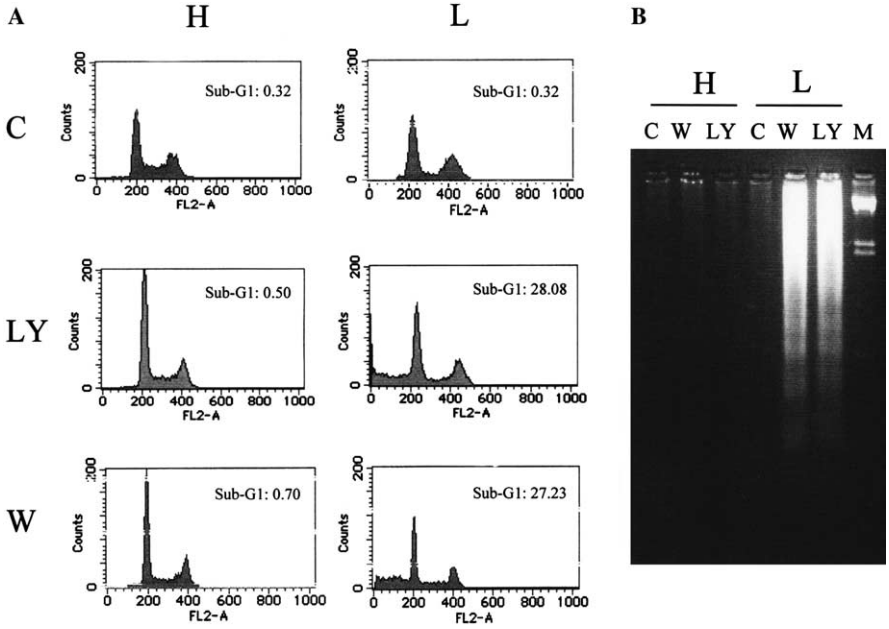

Fig. 1. PI3K inhibitors modulated P53-induced cell cycle arrest to apoptosis. S138V cells were treated with 10 μM LY294002 (LY), 0.5 μM wortmannin (W) or vehicle (C) for 1 h at 37.5 °C (H). Then, the cells were either shifted to 32.5 °C (L) or maintained in culture at 37.5 °C. After 24 h, the cells were collected for FACS (A) or DNA fragmentation analysis (B).

apoptosis, which was dependent on the wild-type activity of p53.

PI3K inhibitor induced apoptosis in growth-arrested cells

Although the above experiment was conducted using growing cells which were treated with PI3K inhibitors, followed by temperature shift-down, the cells were first shifted down for 18 h and then treated with inhibitors to study whether or not PI3K inhibitors can change the cellular response of growth-arrested S138V cells to apoptosis. As shown in Fig. 2A, cells with typical apoptotic bodies were observed with the PI3K inhibitor treatment. A number of cells with apoptotic bodies were observed readily at 12 h and increased to at least 24 h, while the response to the MEK inhibitor was minimum (Fig. 2B).

Phosphorylation levels of Akt and ERK in apoptotic cells

It has been reported that both phosphorylated (activated) forms of Akt and ERK 1/2 play important roles in cell survival. The phosphorylation levels of both proteins were thus first compared using phospho-specific anti-Akt (phospho-473) and anti-ERK1/2 antibodies, before and after the temperature shift-down treatment without addition of inhibitors. Fig. 3 shows that the growth arrest enhanced the phosphorylation levels of both proteins, being favorable for cell survival at the permissive temperature. However, by the additional treatment with LY294002 or wortmannin for 24 h, phospho-Akt was hardly detectable in the cells incubated at the permissive temperature, while the levels were reduced at the non-permissive temperature. In contrast, the phospho-AKT levels changed little in the cells treated with U0126, although the treatment with U0126 effectively blocked ERK1/2 phosphorylation at both temperatures. These results suggest the possibility that loss of phosphorylation of Akt at codon 473 is more important than that of ERK1/2 in changing the S138V cell response from cell growth arrest to apoptosis. In Fig. 3, we also analyzed the $p21^{waf1}$ levels induced at low temperature, but they were essentially the same in cells treated or not treated with PI3K or MEK inhibitor, suggesting that the apoptosis induction was not correlated with the $p21^{waf1}$ level.

Cytosolic release of cytochrome c, bax translocation to the mitochondria, and cleavage of caspases 3 and 9

To study how the PI3K inhibitors led to apoptosis of the growth-arrested S138V cells, we analyzed the intracellular signaling of apoptosis. First, we examined the activation of caspases 3 and 9. Fig. 4A shows that the cleaved and active forms were observed at 24 h after inhibitor addition, and cleaved PARP, a caspase 3 substrate, was also observed. With U0126 or without the inhibitor treatment, the cleavage was not significantly detected or minimum at either temperature.

Next, the translocation of cytochrome *c* into the cytosol from the non-cytosolic mitochondria-containing fraction was examined (Fig. 4B). Without inhibitor

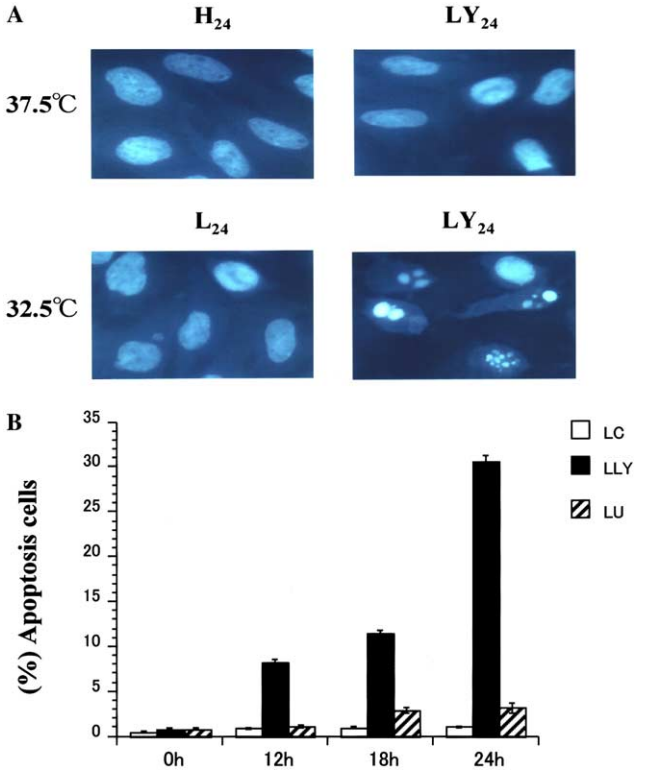

Fig. 2. LY treatment induced apoptosis in growth-arrested S138V cells. (A) S138V cells (1×10^4) were seeded into a 12-well plate and cultured at either 37.5 or 32.5 °C for 18 h. Then, the cells were incubated with (LY24) or without LY294002 (H24 or L24). After 24 h, the cells were fixed for 3 min with ice-cold 1:1 methanol/acetone, then stained with Hoechst 33342 (40 μg/ml), and observed under a fluorescence microscope. (B) S138V cells were grown, cultured, and treated with 10 μM LY294002 (LLY), U0126 (LU) or vehicle (LC), and after various times the cells were stained as in (A). The percentage of cells with pyknotic nuclei (% apoptotic cells) was determined. Data represent means ± SD of three independent experiments.

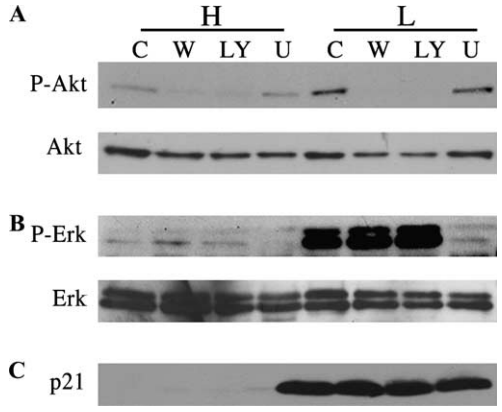

Fig. 3. Enhanced phosphorylation of Akt and Erk1/2 upon p53 activation and Akt down-regulation by treatment with PI3K inhibitors. S138V cells were cultured first at 32.5 °C (L) or at 37.5 °C (H) for 18 h and then treated with 10 μM LY294002 (LY), 0.5 μM wortmannin (W), 10 μM U0126 (U) or vehicle (C) for 24 h at each temperature. Proteins were then extracted and subjected to Western blotting using antibodies against (A) phopho-Akt and Akt, (B) phospho-ERK1/2 and ERK1/2, and (C) $p21^{waf1}$ as described in Materials and methods.

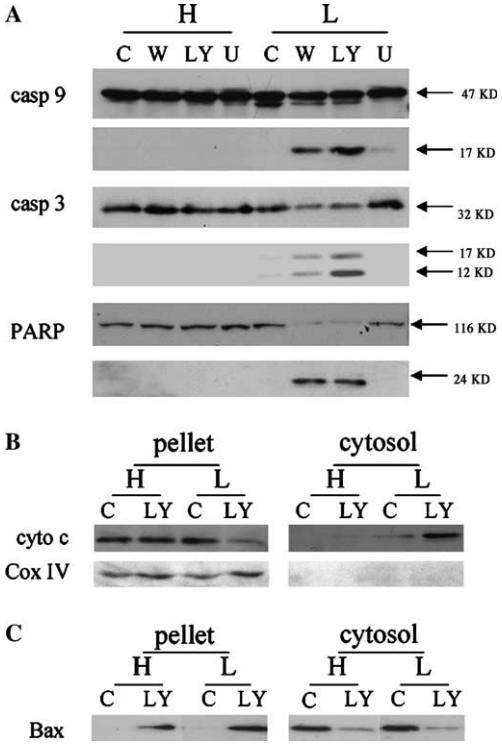

Fig. 4. Activation of caspases 9 and 3, and cytochrome *c* release and Bax translocation to the mitochondria upon LY294002 treatment. S138V cells were incubated with LY294002 as described in Fig. 3. Proteins were extracted after 24 h of treatment and analyzed by Western blotting. (A) The whole cell extracts were analyzed for caspases 9 and 3. The arrows are cleaved forms. (B,C) After LY294002 treatment, the cytosolic fraction was separated from the non-cytosolic fraction containing the mitochondria. Each fraction was probed with (B) anti-cytochrome *c* and anti-Cox IV antibodies, and (C) anti-Bax antibody.

treatment, a small amount of cytochrome *c* was released into the cytosol in cells incubated at the permissive temperature for 18 h, but not at the non-permissive temperature. Upon treatment with the inhibitor, most of the cytochrome *c* was released to the cytosol. On the contrary, no significant amount of Bax was detected in the non-cytosolic fraction of cells incubated at both temperatures, but the inhibitor treatment induced translocation of most of Bax from the cytosol to the non-cytosolic mitochondria-containing fraction. The cytochrome *c* oxidase (COX) sub-unit IV, which was located in the mitochondria, was not released to the cytosol.

These results altogether suggest that the blocking of the PI3K/Akt pathway played an important role in the induction of wild-type p53 activity-dependent apoptosis.

Discussion

The p53 tumor suppressor protein plays a crucial role in regulating cell growth following exposure to various stress stimuli, which resulted in growth arrest

or apoptosis, depending on the final integration of incoming signals [23,24].

In this report, we studied the cellular response of *ts*-p53 expressing S138V cells and found that the response was changed from growth arrest to apoptosis, which required the wild-type activity of p53 and inhibition of the PI3K/Akt pathway, but not that of the ERK pathway. P53-dependent apoptosis is mostly mediated by transcriptionally regulated target proteins that exert their effects either through the mitochondrial pathway or through the death-receptor pathway [2]. In the growth-arrested S138V cells, it is anticipated that p53-target proteins involved in apoptosis induction as well as proteins in growth arrest are up- or down-regulated before the addition of LY294002. The PI3K inhibitors were thus sufficient to trigger the induction of apoptosis by modulating proteins working in the pro-apoptotic or anti-apoptotic pathway, and hence further transactivation function of p53 might not be necessary.

PI3K inhibitors induced dephosphorylation of phospho 473-Akt but not ERK1/2. It has been reported that activated phospho Akt protects the cells from apoptosis by suppressing (a) Bax translocation to the mitochondria [25] and (b) caspase 9 cleavage. Caspase 9 was reported to be directly phosphorylated by activated Akt and the pro-apoptotic function was suppressed [26–28], although the Akt-dependent mechanism of Bax translocation to the mitochondria is not known in detail. After LY294002 treatment of S138V cells, the cleavage of caspase 9 and Bax translocation to the mitochondria were observed. These results taken together suggest that the blocking of the PI3K/Akt survival pathway induced apoptosis in S138V cells when p53 status was wild-type. It should be noted that low levels of Bax translocation to the mitochondria were also observed in LY294002-treated cells at the non-permissive temperature, thus suggesting that translocation of the pro-apoptotic Bcl-2 family protein may be necessary but not sufficient for the induction of apoptosis in S138V cells.

We observed a small amount of cytochrome *c* released into the cytosol in the growth-arrested S138V cells but not in growing cells. While cytochrome *c* release is reported to activate caspase 9 and the subsequent cascade [10], it is also reported that microinjection of cytochrome *c* did not induce apoptosis in a cancer cell line, LNCaP [31]. This discrepancy is explained either by (a) caspase 9 is inactivated by phosphorylation with Akt as described above, or (b) IAP family proteins suppress caspase 9 activation [29]. Since we could not detect cleaved caspases 3, 9 and PARP without the PI3K inhibitors, cytosolic release of cytochrome *c* is thus possible not to have activated caspase 9.

Based on our results and the reports of others, we propose a model for *ts*-p53-induced cell cycle arrest or apoptosis in Saos-2 cells (Fig. 5). The temperature shift-down induces transcriptional activation of $p21^{waf1/cip1}$ and the N-terminal truncated form of p130 (S-p130), both of which along with the intact p130 participate in the inhibition of cyclin-dependent kinase, resulting in cell cycle arrest by p53 [22]. The shift-down also enhances the levels of activated forms of the anti-apoptotic protein p-Akt blocking the activation of caspase 9 and Bax translocation to the mitochondria. The PI3K inhibitors release this block, leading to the induction of apoptosis. In this model, the balance between the levels of growth and apoptosis signals is controlled by PI3K/Akt pathway which plays a crucial role in determining the cell fate. Since the same *ts*-p53 was reported to induce apoptosis in Ewing tumor cells [30] and Jurkat cells [20] under similar conditions used for Saos-2 cells at the permissive temperature without PI3K inhibitor treatment, it is also possible that additional factor(s) may be involved in controlling the balance.

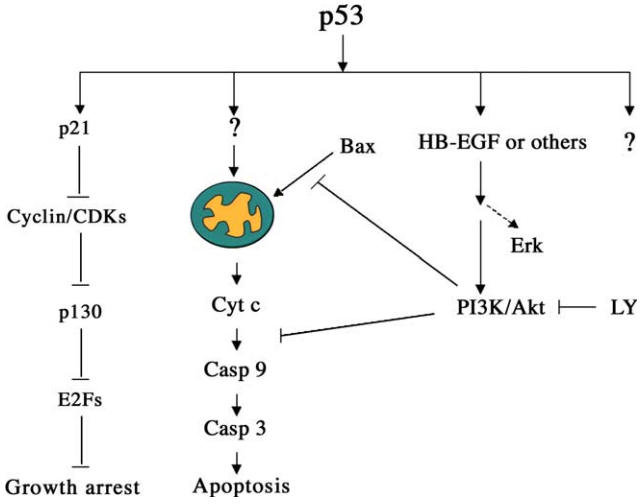

Fig. 5. Schematic model of p53-mediated growth controlling the signal pathways to growth arrest [19] and to apoptosis in Saos-2 cells. The model shows that the PI3K pathway modulates the apoptosis pathway, leading to cell survival.

In conclusion, we showed that the PI3K/Akt pathway plays a crucial role in controlling whether cells go into *ts* p53-induced cell cycle arrest or undergo apoptosis in Saos-2 cells. We succeeded for the first time in modulating these two important effects induced by p53, namely cell cycle arrest and apoptosis, in a single system. This finding may point to a novel aspect of PI3K/Akt function and provide an insight into a chemotherapeutic approach when cancer cells are in growth arrest.

References

[1] M. Oren, Decision making by p53: life, death and cancer, Cell Death Differ. 10 (2003) 431–442.

[2] K.H. Vousden, X. Lu, Live or let die: the cell's response to p53, Nat. Rev. Cancer 2 (2002) 594–604.
[3] C.J. Sherr, J.M. Roberts, CDK inhibitors: positive and negative regulators of G1-phase progression, Genes Dev. 13 (1999) 1501–1512.
[4] T.A. Chan, P.M. Hwang, H. Hermeking, K.W. Kinzler, B. Vogelstein, Cooperative effects of genes controlling the G(2)/M checkpoint, Genes Dev. 14 (2000) 1584–1588.
[5] T. Miyashita, J.C. Reed, Tumor suppressor p53 is a direct transcriptional activator of the human bax gene, Cell 80 (1995) 293–299.
[6] E. Oda, R. Ohki, H. Murasawa, J. Nemoto, T. Shibue, T. Yamashita, T. Tokino, T. Taniguchi, N. Tanaka, Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis, Science 288 (2000) 1053–1058.
[7] K. Nakano, K.H. Vousden, PUMA, a novel proapoptotic gene, is induced by p53, Mol. Cell. 7 (2001) 683–694.
[8] K. Matsuda, K. Yoshida, Y. Taya, K. Nakamura, Y. Nakamura, H. Arakawa, p53AIP1 regulates the mitochondrial apoptotic pathway, Nat. Cell Biol. 5 (2003) 216–223.
[9] R.M. Kluck, E. Bossy-Wetzel, D.R. Green, D.D. Newmeyer, The release of cytochrome *c* from mitochondria: a primary site for Bcl-2 regulation of apoptosis, Science 275 (1997) 1132–1136.
[10] D.R. Green, J.C. Reed, Mitochondria and apoptosis, Science 281 (1998) 1309–1312.
[11] P. Li, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri, X. Wang, Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, Cell 91 (1997) 479–489.
[12] S.R. Datta, A. Brunet, M.E. Greenberg, Cellular survival: a play in three Akts, Genes Dev. 13 (1999) 2905–2927.
[13] A. Bonni, A. Brunet, A.E. West, S.R. Datta, M.A. Takasu, M.E. Greenberg, Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms, Science 286 (1999) 1358–1362.
[14] A. Brunet, S.R. Datta, M.E. Greenberg, Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway, Curr. Opin. Neurobiol. 11 (2001) 297–305.
[15] P. Erhardt, E.J. Schremser, G.M. Cooper, B-Raf inhibits programmed cell death downstream of cytochrome *c* release from mitochondria by activating the MEK/Erk pathway, Mol. Cell. Biol. 19 (1999) 5308–5315.
[16] R. Ley, K. Balmanno, K. Hadfield, C. Weston, S.J. Cook, Activation of the ERK-1/2 signalling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only Bim, J. Biol. Chem. 278 (2003) 18811–18816.
[17] L. Simpson, R. Parsons, PTEN: life as a tumor suppressor, Exp. Cell Res. 264 (2001) 29–41.
[18] L. Fang, G. Li, G. Liu, S.W. Lee, S.A. Aaronson, p53 induction of heparin-binding EGF-like growth factor counteracts p53 growth suppression through activation of MAPK and PI3K/Akt signaling cascades, EMBO J. 20 (2001) 1931–1939.
[19] C.F. Gao, S. Ren, J. Wang, S.L. Zhang, F. Jin, T. Nakajima, M. Ikeda, N. Tsuchida, P130 and its truncated form mediate p53-induced cell cycle arrest in Rb(−/−) Saos2 cells, Oncogene 21 (2002) 7569–7579.
[20] K. Yamato, M. Yamamoto, Y. Hirano, N. Tsuchida, Oncogene 11 (1995) 1–6.
[21] A.R Gottlieb, D.J. Granville, Analyzing mitochondrial changes during apoptosis, Methods 26 (2002) 341–347.
[22] C.F. Gao, S. Ren, L. Zhang, T. Nakajima, S. Ichinose, T. Hara, K. Koike, N. Tsuchida, Caspase-dependent cytosolic release of cytochrome *c* and membrane translocation of Bax in p53-induced apoptosis, Exp. Cell Res. 265 (2001) 145–151.
[23] R.V. Sionov, Y. Haupt, The cellular response to p53: the decision between life and death, Oncogene 18 (1999) 6145–6157.
[24] K.H. Vousden, p53: death star, Cell 103 (2000) 691–694.
[25] F. Tsuruta, N. Masuyama, Y. Gotoh, The phosphatidylinositol 3-kinase (PI3K)-Akt pathway suppresses Bax translocation to mitochondria, J. Biol. Chem. 277 (2002) 14040–14047.
[26] L. del Peso, M. Gonzalez-Garcia, C. Page, R. Herrera, G. Nunez, Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt, Science 278 (1997) 687–689.
[27] S.R. Datta, H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh, M.E. Greenberg, Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery, Cell 91 (1997) 231–241.
[28] M.H. Cardone, N. Roy, H.R. Stennicke, G.S. Salvesen, T.F. Franke, E. Stanbridge, S. Frisch, J.C. Reed, Regulation of cell death protease caspase-9 by phosphorylation, Science 282 (1998) 1318–1321.
[29] G.S. Salvesen, C.S. Duckett, IAP proteins: blocking the road to death's door, Nat. Rev. Mol. Cell. Biol. 3 (2002) 401–410.
[30] H. Kovar, G. Jug, D. Printz, S. Bartl, G. Schmid, J. Wesierska-Gadek, Characterization of distinct consecutive phases in non-genotoxic p53-induced apoptosis of Ewing tumor cells and the rate-limiting role of caspase 8, Oncogene 19 (2000) 4096–4107.
[31] J.P. Carson, M. Behnam, J.N. Sutton, C. Du, X. Wang, D.F. Hunt, M.J. Weber, G. Kulik, Smac is required for cytochrome *c*-induced apoptosis in prostate cancer LNCaP cells, Cancer Res. 62 (2002) 18–23.