# AKT/mTOR Down-regulation by CX-4945, a CK2 Inhibitor, Promotes Apoptosis in Chemorefractory Non-small Cell Lung Cancer Cells

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**Abstract**. *Aim: The response to chemotherapeutic drugs in non-small cell lung cancer (NSCLC) is unsatisfactory, leading to poor outcomes. This study the aimed to investigates anticancer effects of CX-4945, a potent casein kinase II (CK2) inhibitor, in chemorefractory NSCLC cells. Materials and Methods: Cell proliferation and apoptosis assay were carried-out by annexin V-FITC and FACScan after drug treatment with paclitaxel, cisplatin and CX-4945. AKT/mTOR and CK2α signals were measured by western blotting. Treatment was carried-out using siRNA to inhibit CK2α. Results: Paclitaxel, and cisplatin effectively inhibited cell proliferation and induced apoptosis in A549 cells, while not in H1299, Calu-1 and H358 cells. In these chemorefractory cell lines, AKT signalling was maintained despite drug treatment. However, CX-4945 suppressed cell growth, with cell-cycle arrest at $G_2$/M phase and induced apoptosis with an increase of cleaved caspase-3 and PARP1 in a dose-dependent manner. Accordingly, AKT and its downstream signals such as mTOR and p70S6K were down-regulated by CX-4945. Transfection of CK2α siRNA had similar effects to CX-4945 treatment on cell proliferation and apoptosis. Conclusion: CX-4945 shows a promising anticancer action through down-regulation of AKT/mTOR signals, suggesting its possible application for treatment of chemorefractory lung cancer.*

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Lung cancer is the leading cause of cancer-related death in the world, and non-small cell lung cancer (NSCLC) accounts for approximately 80% of all cases (1, 2). Despite advances in diagnostic and therapeutic technology, the overall 5-year survival rate in many countries is generally less than 15% (7). *Cis*-diaminodichloroplatinum (II) (cisplatin) and paclitaxel (a taxol) are the anticancer drugs widely used for the treatment of various types of human cancers, including lung cancer (25). However, the primary resistance or the ability of cancer cells to become resistant to a drug remains a significant problem in successful chemotherapy.

The PI3K/AKT/mTOR signaling pathway plays a significant role in regulating cell survival, cell cycle and apoptosis (12). Many types of cancers, including lung cancer, are known to abnormally activate this pathway (22, 29). Recent studies have shown that the PI3K/AKT/mTOR pathway is one of important causative factors for cancer cells to become resistant to platinum-based chemotherapy (8, 13, 16). Furthermore, the sensitivity of cancer cells to drugs was restored by LY294002, a small-molecule inhibitor of PI3K (3). Taken together, these findings indicate that this pathway could be a promising therapeutic target in cancer management.

Protein kinase CK2 is a ubiquitous serine/threonine kinase involved in cell signaling related to cell-cycle progression, proliferation, and apoptosis. CK2 is present as a tetramer composed of two catalytic subunits and regulatory subunits (9, 20, 23). Aberrant CK2 expression has been reported in a variety of cancer types (28). The overexpression of CK2 in cancer cells has an anti-apoptotic and pro-survival effect. In contrast, its down-regulation enhances cell death caused by drugs or radiation. This suggests that CK2 may have an important role in determining cancer-cell fate (1, 19, 26). Interestingly, inhibition of CK2 activity by a selective inhibitor or CK2 knockdown by siRNA suppressed activation of the PI3K/AKT/mTOR pathway and downstream gene expression (33). Furthermore, phosphorylation of PTEN, which is a tumor suppressor through negative regulation of the PI3K pathway,

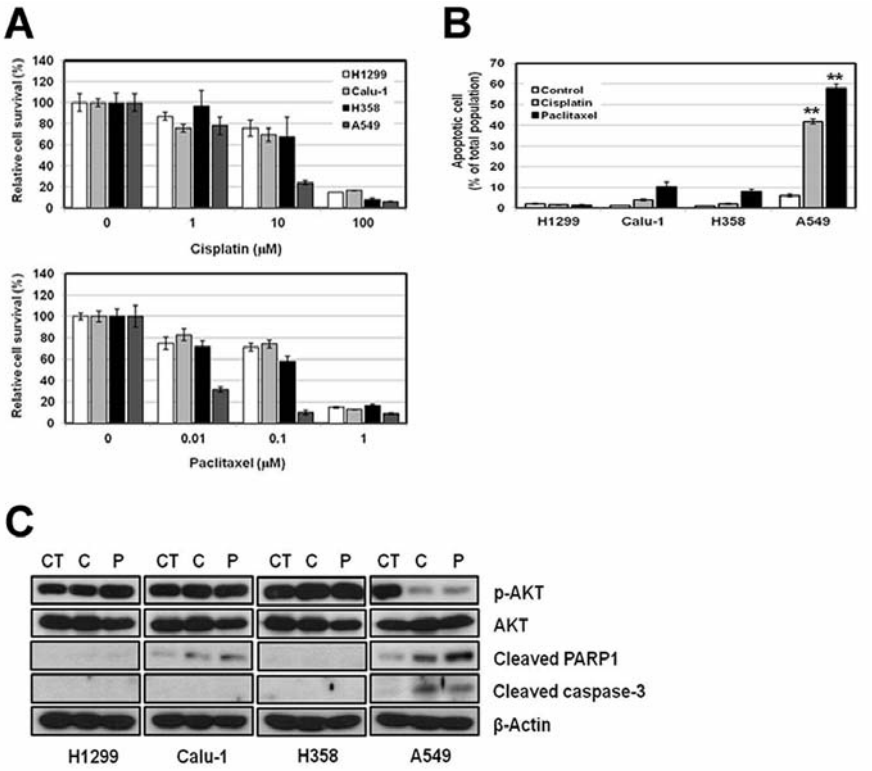


Figure 1. *Expression of phospho-AKT is maintained in the chemotherapy-resistant cell lines. A: Cells were treated with cisplatin and paclitaxel for 72 h in a dose-dependent manner. Cell numbers were determined using an ADAM-MC automatic cell counter. B: Apoptosis was assessed by annexin V-FITC/propidium iodide staining and flow cytometry. **p<0.001 compared to the control (CT). C: Cisplatin, P: paclitaxel. C: Levels of phospho-AKT and AKT, and cleavage of PARP1 and caspase-3 as shown by western blot analysis.*

by CK2 is known to inhibit PTEN activity, leading to increased survival for cancer cells (6), suggesting possible links between CK2 and PI3K/AKT/mTOR pathway in cancer.

In the present study, we evaluated the anticancer effects of CX-4945, a selective and potent CK2 inhibitor, on chemorefractory NSCLC cells.

## Materials and Methods

*Cell cultures and reagents*. The human NSCLC cell lines A549, H1299, Calu-1 and H358 were obtained from the American Type Culture Collection (Rockville, MD, USA) and were cultured in RPMI-1640 (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen) at 37˚C in an atmosphere with 5% $CO_2$. Paclitaxel and cisplatin were purchased from Sigma (St. Louis, MO, USA) and CX-4945 was purchased from Selleck Chemicals Co. Ltd (Houston, TX, USA).

*Cell survival assays*. Cells ($5\times10^5$ and $1\times10^6$) were seeded into a 60 mm dish in triplicate and were treated with the respective agents for 72 h. Cells were trypsinized and cell numbers were determined using an ADAM-MC automatic cell counter (NanoEnTek, Seoul, Korea) according to the manufacturer's instructions.

*Western blot analysis*. Whole-cell lysates were prepared using EBC lysis buffer [50 mM Tris-HCl (pH 8.0), 120 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 0.3 mM phenylmethylsulfonyl fluoride, 0.2 mM sodium orthovanadate, 0.5% NP-40, and 5 U/ml aprotinin] and were then centrifuged. The resulting supernatant (20 μg) was separated on 8% to 12% SDS-PAGE and transferred to PVDF membranes (Invitrogen). The membranes were blocked using 5% skim milk-PBS-0.1% Tween 20 for one hour at room temperature before being incubated overnight with primary antibodies specific for p-CK2α (Sigma), and CK2α (Abcam, Cambridge, UK). Antibodies to AKT, mTOR, p70S6K, caspase-3 and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Those to p-AKT (Ser473), p-mTOR (S2448), p-p70S6K (thr389) and cleaved PARP1 (Asp214) were purchased from Cell Signaling Technology (Berverly, MA, USA). Horseradish peroxidase-conjugated antibodies were used as secondary antibodies. Membranes were developed using ECL kits (PerkinElmer, Waltham, MA, USA).

*Cell-cycle analysis*. Cells were trypsinized, fixed in 70% ethanol at −20˚C from 60 min to a few days, incubated with 5 μl RNase (10 mg/ml) and finally stained with 10 μl propidium iodide (1 mg/ml). The cellular DNA content of treated cells was analyzed by FACScan (Becton Dickinson, Franklin Lakes, NJ, USA).

*Apoptosis assay*. Apoptosis was quantified using Annexin V-FITC

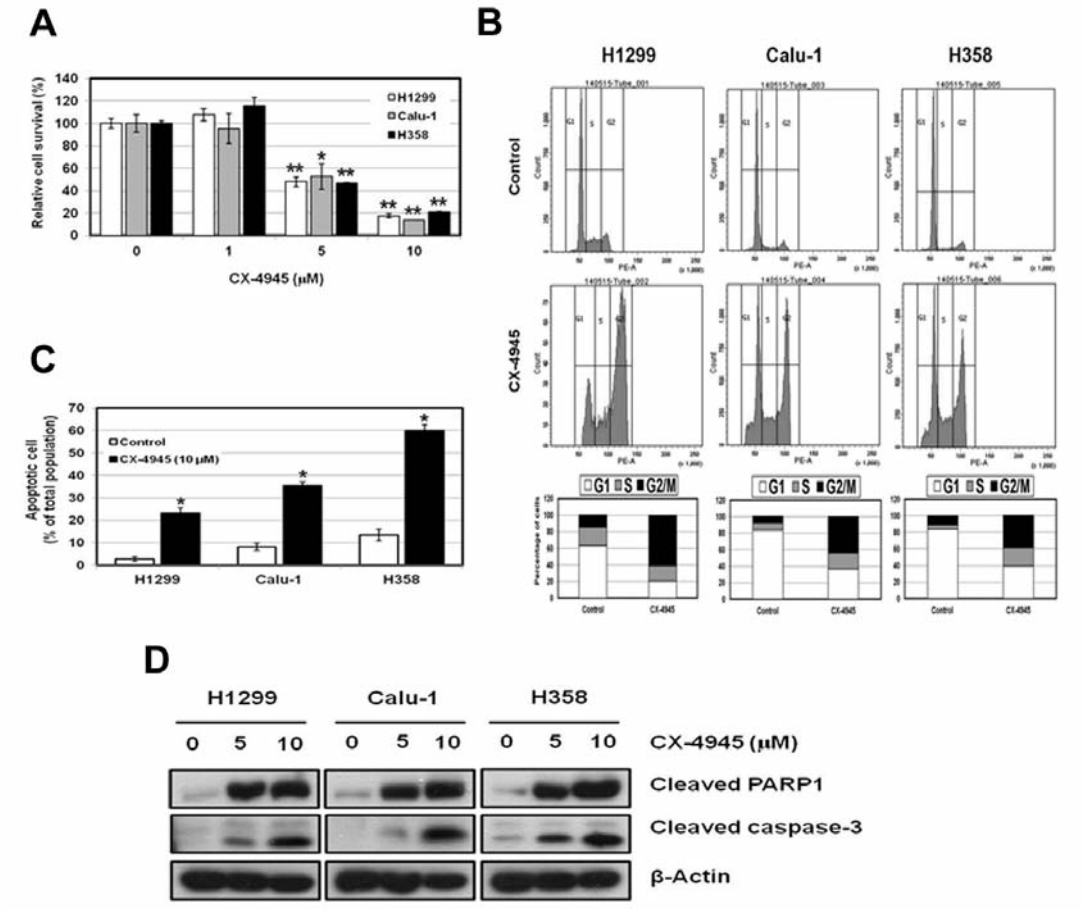


Figure 2. *CX-4945 induced proliferative inhibition, $G_2$/M cell-cycle arrest and apoptosis of chemotherapy-resistant cell lines. A: Cells were treated with CX-4945 (0, 1, 5, 10 μM) for 72 h. Cell numbers were determined using a cell counter. *p<0.01 and **p<0.001 compared to the control. B: Cells were treated with CX-4945 (10 μM) for 48 h. Then the cells were harvested and treated with RNase, and stained with propidium iodide (PI). The cell-cycle distribution was analyzed by flow cytometry. C: Cells were treated with CX-4945 (10 μM) for 72 h. Apoptosis was assessed by annexin V-FITC/PI staining and flow cytometry. *p<0.001 compared to the control. D: Cleavage of PARP1 and caspase-3 as shown by western blot analysis.*

apoptosis kit (BD Biosciences, San Diego, CA, USA) in accordance with the manufacturer's instructions. In brief, cells were trypsinized, pelleted by centrifugation, and resuspended in annexin V binding buffer (150 mM NaCl, 18 mM $CaCl_2$, 10 nM HEPES, 5 mM KCl, 1 mM MgCl2). FITC-conjugated annexin V (1 μg/ml) and propidium iodide (50 μg/ml) was added to the cells which were then incubated for 30 min at room temperature in the dark. Analyses were carried-out on a FACScan instrument (Becton Dickinson). The data were analyzed using CellQuest software (Becton Dickinson). The results were representative of at least three, independent experiments, and the error bars in Figureures signify standard deviations (SDs).

*Small interfering RNA transfection*. Small interfering RNA (siRNA) oligonucleotides specific for CK2α and the siRNA control were purchased from Santa Cruz Biotechnology. Cells were seeded into a 60 mm dish which was then left for 24 h. A 4 μl aliquot of siRNA solution (10 μM) and 10 μl of Lipofectamine 2000 (Invitrogen) were each mixed with 100 μl of serum-free RPMI-1640 medium. These were incubated for 30 min at room temperature after combining the two mixtures, and this was then added to the cells that had been seeded on the dish. After 72 h, harvested cells were counted using an ADAM-MC automatic cell counter (NanaEnTek). Apoptosis was quantified by western blot analysis.

## Results

*AKT phosphorylation was maintained despite cisplatin or paclitaxel treatment in chemorefractory lung cancer cells*. We examined the effect of cisplatin and paclitaxel on the non-small cell lung cancer cell lines H1299, Calu-1, H358 and A549. The cells were treated with increasing concentrations of drugs for 72 h, and the effect of inhibition was determined by a cell counting assay. The $IC_{50}$ values for both cisplatin and paclitaxel against three cell lines (H1299, Calu-1 and H358) were approximately four-to six-times higher than that against the A549 cell line (Figure 1A). When apoptosis was evaluated by

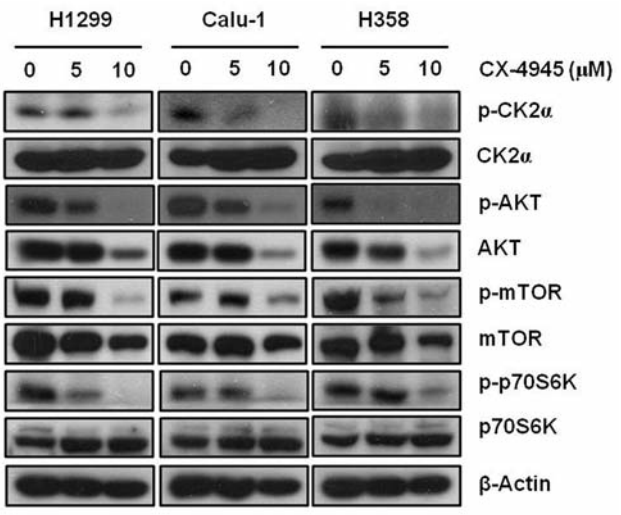


Figure 3. *CX-4945 inhibited the AKT/mTOR signaling molecules in the chemotherapy-resistant cell lines. The three cell lines were treated with CX-4945 (0, 5, 10 μM) for 72 h. Equal amounts of whole-cell lysates (20 μg) were subjected to electrophoresis and the proteins were analyzed by western blotting for phospho (p)-CK2, CK2, p-AKT, AKT, p-mTOR, mTOR, p-p70S6K, p70S6K and β-actin.*

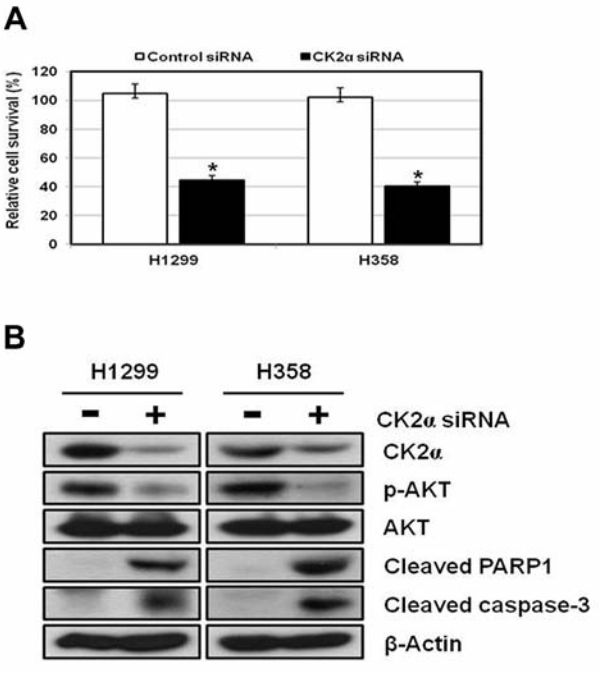


Figure 4. *Suppression of CK2α by siRNA induced apoptosis in the two cell lines studied. A: H1299 and H358 cells were transfected with control siRNA and CK2α siRNA (200 nM). After 72 h, cell viability was measured using a cell counter. *p<0.001 compared to the control. B: The suppression of CK2α, p-AKT and expression of proapoptotic molecules (i.e. cleavage of PARP1 and caspase-3) were detected by western blot analysis.*

flow cytometry after treatment with 10 μM of cisplatin or 100 nM of paclitaxel, the proportion of apoptotic A549 cells was much higher compared to that of the other three cancer cell lines (Figure 1B). Accordingly, cleaved PARP or caspase-3, indicating apoptosis in western blot was induced only in A549 cells (Figure 1C). The activation of AKT was suppressed by drugs in A549 cells, while p-AKT was maintained in the other three chemorefractory cell lines (Figure 1C), suggesting an association between drug response and AKT signal.

*CX-4945 inhibited cell growth through cell-cycle arrest and induced apoptosis of chemorefractory lung cancer cells*. In order investigate the growth-inhibitory effect of CX-4945, chemorefractory cells were treated with CX-4945 for 72 h, and the growth rate was determined by cell counting. CX-4945 effectively suppressed the growth of cancer cells in a dose-dependent manner (Figure 2A). To determine whether CX-4945 inhibited cell-cycle progression of these cell lines, the cell-cycle distribution after treatment with 10 μM of CX-4946 for 72 h was analyzed by flow cytometry. As shown in Figure 2B, the proportion of cells in the $G_2$/M phase was 60.6% in H1299, 43.9% in Calu-1 and 38.8% in H358 cells, respectively. We next observed apoptosis when cells were exposed to CX-4945. After incubation with 10 μM of CX-4945 for 72 h, the cells were analyzed by flow cytometry and western blotting. Early and late apoptotic cells were significantly increased by CX-4945, which was accompanied by increased cleavage of caspase-3 and PARP1 (Figure 2C and D).

*Apoptosis by CX-4945 was related to down-regulation of AKT/mTOR signaling pathway*. In order to better-understand the molecular basis of CX-4945-induced $G_2$/M arrest and apoptosis, we investigated the expression of p-AKT, p-mTOR and downstream signaling molecules after treatment with CX-4945 (0, 5, 10 μM) for 72 h. As shown in Figure 3, the levels of p-AKT, p-mTOR and p-p70S6K dose-dependently decreased in response to CX-4945. Moreover, total AKT protein was also reduced by CX-4945. These results suggest that CX-4945 may be effective for controlling a chemorefractory lung cancer cells through increased $G_2$/M cell-cycle arrest and apoptosis by inhibiton of AKT/mTOR signaling pathway.

*Inhibition of CK2α was required for apoptosis of chemorefractory lung cancer cells*. To determine whether direct inhibition of CK2α in resistant cells is sufficient to induce apoptosis, these cells were transfected with 200 nM of CK2α siRNA. Because the transfection efficiency was very low in Calu-1 cells, two cell lines (H1299 and H358) were used. As shown in Figure 4, treatment with CK2α siRNA suppressed the growth of cancer cells and led to decreased expression of CK2α and p-AKT, as well as increased cleavage of PARP and caspase-3.

## Discussion

Platinum-based chemotherapy is the mainstay of treatment for advanced NSCLC. Although some patients with known driver mutations such as of *EGFR* and *ALK* re-arrangement can benefit from targeted therapy, the majority of patients without those targets should receive cytotoxic chemotherapeutic agents. Despite the development of several new drugs over the past decades, the drug response rate has not improved. Therefore, although a platinum-based doublet of cytotoxic drugs for first-line therapy in patients with good performance status is recommended, more than half of patients do not respond, resulting in a median survival of 8-10 months, with only 5% patients alive at two years (5).

Several factors have been known to be related to therapeutic resistance of cancer cells to anticancer drugs. *P53* mutations are the most common genetic alterations found in human cancer, including lung cancer. Mutations of this gene result in loss of P53 function, contributing to aggressive cancer behavior and drug resistance (15, 18). Three of the cell lines used in our study (H1299, Calu-1, H358) harbor *P53* mutation. This could be one contributing factor to the chemorefractoriness of these cell lines. However, *P53* mutation does not seem to be a major resistance factor in them because CK2 is not related to aberrant *P53*, and CK2 inhibition is effective in controlling these cell lines.

Interestingly, we observed that the activation of AKT was suppressed by cisplatin and paclitaxel in A549 cells, whereas the phospho-AKT was maintained in three chemorefractory cell lines. This suggests that a persistent PI3K/AKT/mTOR pathway can also contribute to the resistance of these cell lines considering that there have been many studies demonstrating its association with drug resistance (8, 13, 16). Accordingly, this pathway was suppressed by the CK2 inhibitor which was able to effectively induce apoptosis.

The induction of cell-cycle arrest and apoptosis are common mechanisms proposed for the cytotoxic effects of anticancer drugs. Cell-cycle arrest can trigger the inhibition of proliferation and increase of apoptosis in cancer cells (4, 21). During the cell cycle, the $G_2$/M checkpoint is a potential target for cancer therapy. It prevents DNA-damaged cells from entering mitosis and allows for the repair of DNA that was damaged in late S or $G_2$ phases prior to mitosis (30). In our study, we observed the increase of cell-cycle arrest at $G_2$/M phase by CX-4945 in chemorefractory cells. This might help cause apoptotic cell death. In line with this, several studies have shown that some anticancer drugs induced $G_2$/M arrest and apoptosis accompanying down-regulation of AKT (2, 11, 31). However, the association of $G_2$/M arrest with PI3K/AKT/mTOR signals should be further explored.

There have been many studies showing that suppression of PI3K/AKT/mTOR signals induces autophagy in cancer cells (14, 24, 32). Rapamycin is a one representative drug which induces autophagy by inhibition of PI3K/AKT/mTOR signaling pathway (27). However, CX-4945 did not cause autophagy in chemorefractory lung cancer cells despite an effective suppression of PI3K/AKT/mTOR signaling pathway. Therefore, autophagy is not linked to the anticancer effects of CX-4945 in our study.

In conclusion, CX-4945 shows promising anticancer effects by down-regulation of AKT/mTOR signals, suggesting its possible application for treatment of chemorefractory lung cancer.

## Conflicts of Interest

We declare that we have no conflicts of interest.

## Acknowledgements

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## References

1 Ahmad KA, Harris NH, Johnson AD, Lindvall HC, Wang G and Ahmed K: Protein kinase CK2 modulates apoptosis induced by resveratrol and epigallocatechin-3-gallate in prostate cancer cells. Molecular cancer therapeutics *6*: 1006-1012, 2007.

2 Asnaghi L, Calastretti A, Bevilacqua A, D'Agnano I, Gatti G, Canti G, Delia D, Capaccioli S and Nicolin A: Bcl-2 phosphorylation and apoptosis activated by damaged microtubules require mTOR and are regulated by Akt. Oncogene *23*: 5781-5791, 2004.

3 Brognard J, Clark AS, Ni Y and Dennis PA: Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. Cancer research *61*: 3986-3997, 2001.

4 Chao JI, Kuo PC and Hsu TS: Down-regulation of survivin in nitric oxide-induced cell growth inhibition and apoptosis of the human lung carcinoma cells. The Journal of biological chemistry *279*: 20267-20276, 2004.

5 Cufer T, Ovcaricek T and O'Brien ME: Systemic therapy of advanced non-small cell lung cancer: major-developments of the last 5-years. European journal of cancer *49*: 1216-1225, 2013.

6 Duncan JS and Litchfield DW: Too much of a good thing: the role of protein kinase CK2 in tumorigenesis and prospects for therapeutic inhibition of CK2. Biochimica et biophysica acta *1784*: 33-47, 2008.

7 Erridge SC, Moller H, Price A and Brewster D: International comparisons of survival from lung cancer: pitfalls and warnings. Nature clinical practice Oncology *4*: 570-577, 2007.

8 Gagnon V, Van Themsche C, Turner S, Leblanc V and Asselin E: Akt and XIAP regulate the sensitivity of human uterine cancer cells to cisplatin, doxorubicin and taxol. Apoptosis: an international journal on programmed cell death *13*: 259-271, 2008.

9 Homma MK and Homma Y: Cell cycle and activation of CK2. Molecular and cellular biochemistry *316*: 49-55, 2008.

10 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, and Thun MJ: Cancer statistics, 2008. CA: a cancer journal for clinicians *58*: 71-96, 2008.

11 Katayama K, Fujita N and Tsuruo T: Akt/protein kinase B-dependent phosphorylation and inactivation of WEE1Hu promote cell cycle progression at $G_2$/M transition. Molecular and cellular biology *25*: 5725-5737, 2005.

12 Kauffmann-Zeh A, Rodriguez-Viciana P, Ulrich E, Gilbert C, Coffer P, Downward J and Evan G: Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. Nature *385*: 544-548, 1997.

13 Kim SH, Juhnn YS and Song YS: Akt involvement in paclitaxel chemoresistance of human ovarian cancer cells. Annals of the New York Academy of Sciences *1095*: 82-89, 2007.

14 Kuo PL, Hsu YL, and Cho CY: Plumbagin induces G2-M arrest and autophagy by inhibiting the AKT/mammalian target of rapamycin pathway in breast cancer cells. Molecular cancer therapeutics *5*: 3209-3221, 2006.

15 Lai SL, Perng RP, and Hwang J: p53 gene status modulates the chemosensitivity of non-small cell lung cancer cells. Journal of biomedical science *7*: 64-70, 2000.

16 Liu LZ, Zhou XD, Qian G, Shi X, Fang J and Jiang BH: AKT1 amplification regulates cisplatin resistance in human lung cancer cells through the mammalian target of rapamycin/p70S6K1 pathway. Cancer research *67*: 6325-6332, 2007.

17 Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA: a cancer journal for clinicians *55*: 74-108, 2005.

18 Perez-Soler R, Kemp B, Wu QP, Mao L, Gomez J, Zeleniuch-Jacquotte A, Yee H, Lee JS, Jagirdar J, and Ling YH: Response and determinants of sensitivity to paclitaxel in human non-small cell lung cancer tumors heterotransplanted in nude mice. Clinical cancer research: an official journal of the American Association for Cancer Research *6*: 4932-4938, 2000.

19 Pierre F, Chua PC, O'Brien SE, Siddiqui-Jain A, Bourbon P, Haddach M, Michaux J, Nagasawa J, Schwaebe MK, Stefan E, Vialettes A, Whitten JP, Chen TK, Darjania L, Stansfield R, Bliesath J, Drygin D, Ho C, Omori M, Proffitt C, Streiner N, Rice WG, Ryckman DM, and Anderes K: Pre-clinical characterization of CX-4945, a potent and selective small molecule inhibitor of CK2 for the treatment of cancer. Molecular and cellular biochemistry *356*: 37-43, 2011.

20 Pinna LA and Meggio F: Protein kinase CK2 ("casein kinase-2") and its implication in cell division and proliferation. Progress in cell cycle research *3*: 77-97, 1997.

21 Pu L, Amoscato AA, Bier ME, and Lazo JS: Dual G1 and G2 phase inhibition by a novel, selective Cdc25 inhibitor 6-chloro-7-[corrected](2-morpholin-4-ylethylamino)-quinoline-5,8-dione. The Journal of biological chemistry *277*: 46877-46885, 2002.

22 Pu X, Hildebrandt MA, Lu C, Lin J, Stewart DJ, Ye Y, Gu J, Spitz MR, and Wu X: PI3K/PTEN/AKT/mTOR pathway genetic variation predicts toxicity and distant progression in lung cancer patients receiving platinum-based chemotherapy. Lung cancer *71*: 82-88, 2011.

23 Ruzzene M and Pinna LA: Addiction to protein kinase CK2: a common denominator of diverse cancer cells? Biochimica et biophysica acta *1804*: 499-504, 2010.

24 Saiki S, Sasazawa Y, Imamichi Y, Kawajiri S, Fujimaki T, Tanida I, Kobayashi H, Sato F, Sato S, Ishikawa K, Imoto M, and Hattori N: Caffeine induces apoptosis by enhancement of autophagy via PI3K/Akt/mTOR/p70S6K inhibition. Autophagy *7*: 176-187, 2011.

25 Siddik ZH: Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene *22*: 7265-7279, 2003.

26 St-Denis NA and Litchfield DW: Protein kinase CK2 in health and disease: From birth to death: the role of protein kinase CK2 in the regulation of cell proliferation and survival. Cellular and molecular life sciences: CMLS *66*: 1817-1829, 2009.

27 Takeuchi H, Kondo Y, Fujiwara K, Kanzawa T, Aoki H, Mills GB and Kondo S: Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. Cancer research *65*: 3336-3346, 2005.

28 Unger GM, Davis AT, Slaton JW and Ahmed K: Protein kinase CK2 as regulator of cell survival: implications for cancer therapy. Current cancer drug targets *4*: 77-84, 2004.

29 Vivanco I and Sawyers CL: The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nature reviews Cancer *2*: 489-501, 2002.

30 Wang Y, Ji P, Liu J, Broaddus RR, Xue F and Zhang W: Centrosome-associated regulators of the G(2)/M checkpoint as targets for cancer therapy. Molecular cancer *8*: 8, 2009.

31 Weir NM, Selvendiran K, Kutala VK, Tong L, Vishwanath S, Rajaram M, Tridandapani S, Anant S and Kuppusamy P: Curcumin induces G2/M arrest and apoptosis in cisplatin-resistant human ovarian cancer cells by modulating Akt and p38 MAPK. Cancer biology & therapy *6*: 178-184, 2007.

32 Wu JC, Lai CS, Badmaev V, Nagabhushanam K, Ho CT and Pan MH: Tetrahydrocurcumin, a major metabolite of curcumin, induced autophagic cell death through coordinative modulation of PI3K/Akt-mTOR and MAPK signaling pathways in human leukemia HL-60 cells. Molecular nutrition & food research *55*: 1646-1654, 2011.

33 Zheng Y, McFarland BC, Drygin D, Yu H, Bellis SL, Kim H, Bredel M and Benveniste EN: Targeting protein kinase CK2 suppresses prosurvival signaling pathways and growth of glioblastoma. Clinical cancer research: an official journal of the American Association for Cancer Research *19*: 6484-6494, 2013.

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