CK2 Inhibition May be a Key Mediator of the Cancer-Retardant Effects of Natural Flavones in Xenografted Nude Mice

Mark F. McCarty, Catalytic Longevity, markfmccarty@gmail.com

Abstract

The serine-threonine kinase CK2, which targets over 300 cellular proteins, is overexpressed in all cancers, presumably reflecting its ability to promote proliferation, spread, and survival through a wide range of complementary mechanisms. Via an activating phosphorylation of Cdc373, a co-chaperone which partners with Hsp90, CK2 prolongs the half-life of protein kinases, including Akt, Src, EGFR, Raf-1, and a range of cyclin-dependent kinases, that promote proliferation and survival in many cancers. And CK2 can work in other ways to boost the activity of a number of signaling pathways that promote cancer aggressiveness and chemoresistance, including those driven by Akt, NF-kappaB, hypoxia-inducible factor-1, beta-catenin, STAT3, and the androgen receptor; it also promotes the epidermal-mesenchymal transition and aids the efficiency of DNA repair. Several potent and relatively specific inhibitors of CK2 are now being evaluated as potential cancer drugs; CX-4945 has shown impressive activity in cell culture studies and xenograft models, either as a stand-alone tumor retardant, or as adjuvant to concurrent chemotherapy, and is now entering clinical trials. However, it has long been recognized that the natural flavone apigenin can inhibit CK2, with a K_i near 1 micromolar; more recent work indicates that a range of flavones and flavonols, characterized by a planar structure and hydroxylations at the 7 and 4' positions – including apigenin, luteolin keampferol, fisetin, quercetin, and myricetin - can inhibit CK2 with K_i s in the sub-micromolar range. This finding is particularly intriguing in light of the numerous studies demonstrating that *each* of these agents can inhibit the growth of cancer cells lines *in vitro* and of human xenografts in nude mice; this essay cites 53 such xenograft studies. These studies attribute the cancer-retardant efficacy of flavones/flavonols to impacts on a bewildering array of cellular targets, including those whose activities are boosted by CK2; it is reasonable to suspect that, at least in physiologically achievable concentrations, these agents may be achieving these effects primarily via CK2 inhibition. Inefficient absorption and rapid conjugation limit the bioefficacy of orally administered flavonoids; however, the increased extracellular beta-glucuronidase of many tumors may give tumors privileged access to glucuronidated flavonoids, and nanopartical technology can improve the bioavailability of these agents. Hence, it may be worthwhile to explore the clinical potential of flavones/flavonols as CK2 inhibitors for cancer therapy.

\

CK2 is Over-expressed and Up-regulates Proliferation, Spread, and Survival in Cancer

CK2, a serine-threonine kinase once known as casein kinase 2, is a ubiquitously expressed tetramer comprised of two catalytic (α and/or α') and two regulatory β subunits. CK2 is capable of phosphorylating a huge range of cellular proteins; over 300 physiological targets have been documented to date (though ironically casein is not one of them!).[1] Its level of expression and its sub-cellular localization determine its activity, as post-translational modifications or allosteric interactions are thought to have little impact in that regard; moreover, no gain-of-function mutants of this kinase are known.

Virtually all cancer cell lines studied to date overexpress CK2 protein, relative to its expression in normal tissues of origin; moreover, they tend to route a higher proportion of this protein to the cell nucleus.[2] This is not likely to be accidental, as high CK2 activity works in a bewildering number of complementary ways to promote cellular proliferation and spread, while suppressing apoptosis. Hence, cancer cells which overexpress CK2 will tend to be selected for.

CK2 Modulates a Plethora of Signaling Pathways

One of CK2's most intriguing and ramified effects is to phosphorylate, and thereby activate, the co-chaperone Cdc37.[3, 4] Activated Cdc37 interacts with Hsp90 to provide chaperoning activity for a broad range of protein kinases, many of which play a role in promoting cell proliferation and survival. These include Akt, Src, EGFR, PDGFR, Raf-1, IKK, RIP1, Cdc2, Cdk2, Cdk4, and Cdk6. This chaperoning activity tends to slow the proteolytic degradation of these kinases, prolonging their effective half-lives; this activity is particularly crucial for the survival of certain mutant constitutively active forms of these kinases often found in cancers. To date, CK2 is the only upstream kinase known to confer activation on Cdc37 – for which reason assessment of Cdc37 phosphorylation at Ser13 has been proposed as a strategy for determining CK2 activity in vivo.[5]

But CK2 works in a number of additional ways to boost the activity of signaling pathways that make cancer more aggressive and harder to kill:

Akt – While CK2 boost Akt expression via Hsp90-cdc37-mediated stabilization, it can also work in various complementary ways to increase the phosphorylation and activation of this key kinase, which promotes cellular proliferation while acting in a number of ways to inhibit apoptosis. CK2 phosphorylates Akt directly at Ser129; this up-regulates the activation of Akt mediated by PDK1 and mTORC2, and facilitates its association with Hsp90.[6, 7] And CK2 inhibits phosphatase activities that target Akt; it phosphorylates and thereby reduces the activity of the crucial cancer suppressor PTEN, and also promotes proteasomal degradation of PML, a protein which is an obligate component of a nuclear complex that dephosphorylates Akt within the nucleus.[8-12] CK2 also has the potential to work upstream from Akt, enhancing its activation by up-regulating certain tyrosine kinase signaling pathways.

NF-kappaB – Numerous studies show that CK2 inhibition suppresses NF-kappaB activity in cancer cell lines, whereas overexpression of this kinase boosts NF-kappaB activity.[13-28] CK2 promotes degradation of IkappaB; this can reflect an activating phosphorylation of IKKbeta, as well as a direct phosphorylation of IkappaB that renders it more sensitive to proteolytic cleavage by calpain.[14, 15, 20, 25] CK2 activity also has been reported to somehow boost the expression of IKK-i/IKKepsilon, an alternative IkappaB kinase complex capable of promoting IkappaB degradation.[18] And the transcriptional activity of p65 is enhanced by a phosphorylation of Ser529 conferred by CK2.[21]

Hypoxia-inducible factor-1 (HIF-1) – CK2 acts to enhance the transcriptional activity of HIF-1, even though it doesn't increase the protein expression or nuclear binding of this factor.[29-31] Some evidence suggests that this reflects a reduction of p53 levels; nuclear p53 somehow antagonizes the transcriptional activity of HIF-1.[30] CK2 impact on p53 level, in turn, may reflect phosphorylations of MDM2 that enhance its ability to promote proteasomal degradation of p53.

Beta-Catenin – Many studies show that CK2 inhibition decrease Wnt-beta-catenin signaling.[23, 32-40] Activation of Akt, which stabilizes beta-catenin through inhibition of glycogen synthase kinase-3 and also via a direct phosphorylation on Ser552, evidently can contribute to this effect.[38, 39] However, CK2 also phosphorylates beta-catenin directly on Thr393, an effect which likewise prolongs the half-life and promotes the transcriptional activity of this factor.[33, 34]

STAT3 – There are several reports that inhibition of CK2 suppresses STAT3 phosphorylation and activation in cancer cell lines.[41-43] The basis of this effect is not yet clear. In some cell lines, suppression of IL-6 expression may contribute to this effect.

Androgen Receptor – CK2 inhibitors suppress androgen receptor-mediated transcription in prostate cancer cell lines, at least in part by blocking androgen-induced nuclear translocation of the receptor.[44-46] The direct target of CK2 in this effect has not been identified.

DNA Repair – CK2-mediated phosphorylations of XRCC1 and MDC1, nuclear proteins which play a key role in the repair of DNA single-strand and double-strand breaks, respectively, are required for their proper activity.[47-52] Hence, inhibition of CK2 can boost the killing activity of DNA-damaging cytotoxins not only by up-regulating mechanisms of apoptosis, but also by impeding the efficiency of DNA repair.

Epidermal-Mesenchymal Transition – Studies with CK2 inhibitors demonstrate that CK2 activity can promote the epidermal-mesenchymal transition necessary for invasive behavior by boosting expression of vimentin, snail, and smad2/3, while suppressing that of E-cadherin.[53-57] For some reason this effect is most prominent in cancer cells which overexpress CK2alpha catalytic subunits, relative to CK2beta regulatory subunits.[58, 59]

New Drugs for Inhibition of CK2 – CX-4945

These considerations make it abundantly clear that well tolerated and effective pharmaceutical inhibitors of CK2 may have a bright future in oncology – both as agents for slowing cancer growth and spread, and as adjuvants to chemo- or radiotherapy. Some pharmaceutical companies are moving aggressively to evaluate the potential of this approach, and the highly potent and orally active CK2 inhibitor CX-4945 has shown impressive anti-cancer activity in mouse xenograft models, in doses which the animals appear to tolerate well.[60, 61] Moreover, in doses that don't greatly retard tumor growth, CX-4945 considerably amplifies response of an ovarian cancer xenograft to gemcitabine and cisplatin – though the somewhat greater weight loss in the mice receiving combination therapy suggests that toxicity might also be increased to a degree.[62] This agent is now entering clinical trials, and its progress should be followed with the greatest interest.

Flavones/Flavonols as Natural Inhibitors of CK2

However, there are other known inhibitors of CK2, one being the dietary flavone apigenin. Indeed, long before the development of the more potent pharmaceutical inhibitors of CK2, this agent was employed as a relatively specific inhibitor of CK2 in cell culture studies, with a K_i near 1 μM.[63] There are indeed a number of studies, both in cancer cell culture and in mouse xenograft models, showing that apigenin can exert cancer-retardant and chemo-potentiating effects. In xenograft models, apigenin has shown activity whether administered parenterally or orally, alone or as an adjuvant to chemotherapy.[64-80] Intriguingly, many of the effects of apigenin on signaling pathways reported in cell culture or xenograft studies are parallel to those of CK2 inhibition, including down-regulated activity of Akt,[4, 73, 81-85] HIF-1,[64, 66, 86-90] NF-kappaB,[4, 18, 22, 29, 91, 91-94] STAT3,[4, 93, 95] beta-catenin,[96, 97] AR,[98, 99] and Cdc37,[4] and up-regulated p53.[93, 100-107] Indeed, Zhao and colleagues have recently proposed that inhibition of CK2 is a key mediator of apigenin's anti-cancer activity in multiple myeloma cells.[4] A survey of the burgeoning cancer research literature involving apigenin – 476 citations on Pubmed at present – reveals apigenin can influence a truly dizzying array of molecular targets in cancer cells; it is reasonable to suspect that, rather than directly inhibiting dozens of separate targets, it must be influencing one or more signaling factors that have a remarkably broad impact on the molecular biology of cancer cells. CK2 may be the crucial target in this regard. However, none of the studies in which apigenin has been administered in cancer-retardant doses to xenograft-bearing mice have assessed the impact of apigenin on CK2 activity. A study assessing this would be worthwhile; and it would also be intriguing to see whether apigenin administration has any significant additional impact on cancer growth in animals that are already receiving potent doses of CX-4945; if CK2 is apigenin's key target, little additional benefit might be seen.

Although apigenin is considered the prototype flavone inhibitor of CK2, recent studies show that other naturally-occuring flavones and flavonols have similar or slightly more potent inhibitory activity. Working in vitro with human recombinant CK2, Lolli and colleagues have recently

reported that apigenin, luteolin, kaempferol, fisetin, quercetin, and myricetin can inhibit CK2 with K_is of 0.8, 0.5, 0.4, 0.35, 0.55, and 0.92 µM, respectively.[108] This inhibition is competitive with respect to the phosphodonor substrate ATP. All effective compouds are planar and are hydroxylated at the7 and 4' positions. Hydroxylations at 5, 3, and 3' positions do not greatly add to or detract from activity.

These findings may help to explain the curious fact that *every one* of these flavones or flavonols has been reported to exert anti-cancer effects, both in cancer cell cultures, and in xenografted mice. Here are citations for the xenograft studies: apigenin,[64-80] luteolin,[109-120] kaempferol,[121] fisetin,[122-124] quercetin,[125-143] myrcetin.[144]

It seems likely that, ultimately, a drug such as CX-4945 will offer the most convenient and effective way to address the CK2 activity of clinical cancer. However, this or comparable drugs will not be available for several years, and when available will initially only be approved for use in a limited number of cancers – and will doubtless be staggeringly expensive to use for off-label purposes. For this reason, it would be prudent to give serious attention to the possibility that apigenin or related flavones/flavonols might be clinically useful for suppressing CK2 activity in some sufficiently high dosage schedule. This might be assessed by pharmacokinetic studies in which a marker for CK2 activity, such as phosphorylation of Cdc37 – or Thr145 phosphorylation of p21, employed as a marker in studies with CX-4549[60] – is determined in leukocytes or some other accessible cell type. The efficacy of a given agent will presumably reflect it absorbability, the rapidity with which it is conjugated once absorbed (glucuronidation or sulfation), and its capacity to pass through cell walls. Pharmaceutical innovations which optimize absorbability might make this approach more feasible.[139]

Rapid conjugation of absorbed flavonoids limits their capacity to exert intracellular effects.[145] It is therefore fortunate that some tumors may have privileged access to flavone/flavonol glucuronide conjugates, owing to the fact that extracellular beta-glucuronidase activity tends to be elevated in tumors, particularly in their hypoxic/necrotic regions.[146, 147] Infiltrating immune cells may be the chief source of this activity. Moreover, the tendency of extracellular pH to be acidic in such regions can be expected to amplify their beta-glucuronidase activity.[148-150] Many investigators have proposed or presented evidence that glucuronide-masked anti-cancer agents – including flavonoids – can be selectively activated within tumors.[147, 150-158] Hence, the rapid glucuronidation of flavones and flavonols may not be an insuperable obstacle to the capacity of these compounds to inhibit CK2 in vivo. Perhaps this mechanism contributes to the demonstrable efficacy of flavones/flavonols in mouse xenograft studies; co-administration of a beta-glucuronidase inhibitor might clarify this. A corollary of this consideration, however, is that measurement of CK2 activity in healthy tissues following oral administration of flavones/flavonols may underestimate the capacity of these agents to inhibit CK2 within tumors.

References

(1) Meggio F, Pinna LA. One-thousand-and-one substrates of protein kinase CK2? *FASEB J* 2003 March;17(3):349-68.

(2) Tawfic S, Yu S, Wang H, Faust R, Davis A, Ahmed K. Protein kinase CK2 signal in neoplasia. *Histol Histopathol* 2001 April;16(2):573-82.

(3) Miyata Y, Nishida E. CK2 controls multiple protein kinases by phosphorylating a kinase-targeting molecular chaperone, Cdc37. *Mol Cell Biol* 2004 May;24(9):4065-74.

(4) Zhao M, Ma J, Zhu HY et al. Apigenin inhibits proliferation and induces apoptosis in human multiple myeloma cells through targeting the trinity of CK2, Cdc37 and Hsp90. *Mol Cancer* 2011;10:104.

(5) Miyata Y, Nishida E. Evaluating CK2 activity with the antibody specific for the CK2-phosphorylated form of a kinase-targeting cochaperone Cdc37. *Mol Cell Biochem* 2008 September;316(1-2):127-34.

(6) Di MG, Brustolon F, Pinna LA, Ruzzene M. Dephosphorylation and inactivation of Akt/PKB is counteracted by protein kinase CK2 in HEK 293T cells. *Cell Mol Life Sci* 2009 October;66(20):3363-73.

(7) Siddiqui-Jain A, Drygin D, Streiner N et al. CX-4945, an orally bioavailable selective inhibitor of protein kinase CK2, inhibits prosurvival and angiogenic signaling and exhibits antitumor efficacy. *Cancer Res* 2010 December 15;70(24):10288-98.

(8) Shehata M, Schnabl S, Demirtas D et al. Reconstitution of PTEN activity by CK2 inhibitors and interference with the PI3-K/Akt cascade counteract the antiapoptotic effect of human stromal cells in chronic lymphocytic leukemia. *Blood* 2010 October 7;116(14):2513-21.

(9) Barata JT. The impact of PTEN regulation by CK2 on PI3K-dependent signaling and leukemia cell survival. *Adv Enzyme Regul* 2011;51(1):37-49.

(10) Kang NI, Yoon HY, Kim HA et al. Protein kinase CK2/PTEN pathway plays a key role in platelet-activating factor-mediated murine anaphylactic shock. *J Immunol* 2011 June 1;186(11):6625-32.

(11) Chatterjee A, Chatterjee U, Ghosh MK. Activation of protein kinase CK2 attenuates FOXO3a functioning in a PML-dependent manner: implications in human prostate cancer. *Cell Death Dis* 2013;4:e543.

(12) Chatterjee A, Chatterjee U, Ghosh MK. Activation of protein kinase CK2 attenuates FOXO3a functioning in a PML-dependent manner: implications in human prostate cancer. *Cell Death Dis* 2013;4:e543.

(13) Romieu-Mourez R, Landesman-Bollag E, Seldin DC, Traish AM, Mercurio F, Sonenshein GE. Roles of IKK kinases and protein kinase CK2 in activation of nuclear factor-kappaB in breast cancer. *Cancer Res* 2001 May 1;61(9):3810-8.

(14) Shen J, Channavajhala P, Seldin DC, Sonenshein GE. Phosphorylation by the protein kinase CK2 promotes calpain-mediated degradation of IkappaBalpha. *J Immunol* 2001 November 1;167(9):4919-25.

(15) Romieu-Mourez R, Landesman-Bollag E, Seldin DC, Sonenshein GE. Protein kinase CK2 promotes aberrant activation of nuclear factor-kappaB, transformed phenotype, and survival of breast cancer cells. *Cancer Res* 2002 November 15;62(22):6770-8.

(16) Kato T, Jr., Delhase M, Hoffmann A, Karin M. CK2 Is a C-Terminal IkappaB Kinase Responsible for NF-kappaB Activation during the UV Response. *Mol Cell* 2003 October;12(4):829-39.

(17) Cavin LG, Romieu-Mourez R, Panta GR et al. Inhibition of CK2 activity by TGF-beta1 promotes IkappaB-alpha protein stabilization and apoptosis of immortalized hepatocytes. *Hepatology* 2003 December;38(6):1540-51.

(18) Eddy SF, Guo S, Demicco EG et al. Inducible IkappaB kinase/IkappaB kinase epsilon expression is induced by CK2 and promotes aberrant nuclear factor-kappaB activation in breast cancer cells. *Cancer Res* 2005 December 15;65(24):11375-83.

(19) Piazza FA, Ruzzene M, Gurrieri C et al. Multiple myeloma cell survival relies on high activity of protein kinase CK2. *Blood* 2006 September 1;108(5):1698-707.

(20) Yu M, Yeh J, Van WC. Protein kinase casein kinase 2 mediates inhibitor-kappaB kinase and aberrant nuclear factor-kappaB activation by serum factor(s) in head and neck squamous carcinoma cells. *Cancer Res* 2006 July 1;66(13):6722-31.

(21) Parhar K, Morse J, Salh B. The role of protein kinase CK2 in intestinal epithelial cell inflammatory signaling. *Int J Colorectal Dis* 2007 June;22(6):601-9.

(22) Hamacher R, Saur D, Fritsch R, Reichert M, Schmid RM, Schneider G. Casein kinase II inhibition induces apoptosis in pancreatic cancer cells. *Oncol Rep* 2007 September;18(3):695-701.

(23) Dominguez I, Sonenshein GE, Seldin DC. Protein kinase CK2 in health and disease: CK2 and its role in Wnt and NF-kappaB signaling: linking development and cancer. *Cell Mol Life Sci* 2009 June;66(11-12):1850-7.

(24) Brown MS, Diallo OT, Hu M et al. CK2 modulation of NF-kappaB, TP53, and the malignant phenotype in head and neck cancer by anti-CK2 oligonucleotides in vitro or in vivo via sub-50-nm nanocapsules. *Clin Cancer Res* 2010 April 15;16(8):2295-307.

(25) Tsuchiya Y, Asano T, Nakayama K, Kato T, Jr., Karin M, Kamata H. Nuclear IKKbeta is an adaptor protein for IkappaBalpha ubiquitination and degradation in UV-induced NF-kappaB activation. *Mol Cell* 2010 August 27;39(4):570-82.

(26) Trembley JH, Unger GM, Tobolt DK et al. Systemic administration of antisense oligonucleotides simultaneously targeting CK2alpha and alpha' subunits reduces orthotopic xenograft prostate tumors in mice. *Mol Cell Biochem* 2011 October;356(1-2):21-35.

(27)   Trembley JH, Unger GM, Korman VL et al. Nanoencapsulated anti-CK2 small molecule drug or siRNA specifically targets malignant cancer but not benign cells. *Cancer Lett* 2012 February 1;315(1):48-58.

(28)   Zheng Y, McFarland BC, Drygin D et al. Targeting Protein Kinase CK2 Suppresses Pro-survival Signaling Pathways and Growth of Glioblastoma. *Clin Cancer Res* 2013 September 13.

(29)   Mottet D, Ruys SP, Demazy C, Raes M, Michiels C. Role for casein kinase 2 in the regulation of HIF-1 activity. *Int J Cancer* 2005 December 10;117(5):764-74.

(30)   Hubert A, Paris S, Piret JP, Ninane N, Raes M, Michiels C. Casein kinase 2 inhibition decreases hypoxia-inducible factor-1 activity under hypoxia through elevated p53 protein level. *J Cell Sci* 2006 August 15;119(Pt 16):3351-62.

(31)   Ampofo E, Kietzmann T, Zimmer A, Jakupovic M, Montenarh M, Gotz C. Phosphorylation of the von Hippel-Lindau protein (VHL) by protein kinase CK2 reduces its protein stability and affects p53 and HIF-1alpha mediated transcription. *Int J Biochem Cell Biol* 2010 October;42(10):1729-35.

(32)   Song DH, Sussman DJ, Seldin DC. Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. *J Biol Chem* 2000 August 4;275(31):23790-7.

(33)   Song DH, Dominguez I, Mizuno J, Kaut M, Mohr SC, Seldin DC. CK2 phosphorylation of the armadillo repeat region of beta-catenin potentiates Wnt signaling. *J Biol Chem* 2003 June 27;278(26):24018-25.

(34)   Seldin DC, Landesman-Bollag E, Farago M, Currier N, Lou D, Dominguez I. CK2 as a positive regulator of Wnt signalling and tumourigenesis. *Mol Cell Biochem* 2005 June;274(1-2):63-7.

(35)   Tapia JC, Torres VA, Rodriguez DA, Leyton L, Quest AF. Casein kinase 2 (CK2) increases survivin expression via enhanced beta-catenin-T cell factor/lymphoid enhancer binding factor-dependent transcription. *Proc Natl Acad Sci U S A* 2006 October 10;103(41):15079-84.

(36)   Wang S, Jones KA. CK2 controls the recruitment of Wnt regulators to target genes in vivo. *Curr Biol* 2006 November 21;16(22):2239-44.

(37)   Lee AK, Ahn SG, Yoon JH, Kim SA. Sox4 stimulates ss-catenin activity through induction of CK2. *Oncol Rep* 2011 February;25(2):559-65.

(38)   Ponce DP, Maturana JL, Cabello P et al. Phosphorylation of AKT/PKB by CK2 is necessary for the AKT-dependent up-regulation of beta-catenin transcriptional activity. *J Cell Physiol* 2011 July;226(7):1953-9.

(39)   Ponce DP, Yefi R, Cabello P et al. CK2 functionally interacts with AKT/PKB to promote the beta-catenin-dependent expression of survivin and enhance cell survival. *Mol Cell Biochem* 2011 October;356(1-2):127-32.

(40)   Kim J, Hwan KS. CK2 Inhibitor CX-4945 Blocks TGF-beta1-Induced Epithelial-to-Mesenchymal Transition in A549 Human Lung Adenocarcinoma Cells. *PLoS ONE* 2013;8(9):e74342.

(41) Piazza FA, Ruzzene M, Gurrieri C et al. Multiple myeloma cell survival relies on high activity of protein kinase CK2. *Blood* 2006 September 1;108(5):1698-707.

(42) Lin YC, Hung MS, Lin CK et al. CK2 inhibitors enhance the radiosensitivity of human non-small cell lung cancer cells through inhibition of stat3 activation. *Cancer Biother Radiopharm* 2011 June;26(3):381-8.

(43) Zhao M, Ma J, Zhu HY et al. Apigenin inhibits proliferation and induces apoptosis in human multiple myeloma cells through targeting the trinity of CK2, Cdc37 and Hsp90. *Mol Cancer* 2011;10:104.

(44) Ryu BJ, Baek SH, Kim J et al. Anti-androgen receptor activity of apoptotic CK2 inhibitor CX4945 in human prostate cancer LNCap cells. *Bioorg Med Chem Lett* 2012 September 1;22(17):5470-4.

(45) Yao K, Youn H, Gao X et al. Casein kinase 2 inhibition attenuates androgen receptor function and cell proliferation in prostate cancer cells. *Prostate* 2012 September 15;72(13):1423-30.

(46) Gotz C, Bachmann C, Montenarh M. Inhibition of protein kinase CK2 leads to a modulation of androgen receptor dependent transcription in prostate cancer cells. *Prostate* 2007 February 1;67(2):125-34.

(47) Loizou JI, El-Khamisy SF, Zlatanou A et al. The protein kinase CK2 facilitates repair of chromosomal DNA single-strand breaks. *Cell* 2004 April 2;117(1):17-28.

(48) Parsons JL, Dianova II, Finch D et al. XRCC1 phosphorylation by CK2 is required for its stability and efficient DNA repair. *DNA Repair (Amst)* 2010 July 1;9(7):835-41.

(49) Strom CE, Mortusewicz O, Finch D et al. CK2 phosphorylation of XRCC1 facilitates dissociation from DNA and single-strand break formation during base excision repair. *DNA Repair (Amst)* 2011 September 5;10(9):961-9.

(50) Siddiqui-Jain A, Bliesath J, Macalino D et al. CK2 inhibitor CX-4945 suppresses DNA repair response triggered by DNA-targeted anticancer drugs and augments efficacy: mechanistic rationale for drug combination therapy. *Mol Cancer Ther* 2012 April;11(4):994-1005.

(51) Chapman JR, Jackson SP. Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. *EMBO Rep* 2008 August;9(8):795-801.

(52) Becherel OJ, Jakob B, Cherry AL et al. CK2 phosphorylation-dependent interaction between aprataxin and MDC1 in the DNA damage response. *Nucleic Acids Res* 2010 March;38(5):1489-503.

(53) Belguise K, Guo S, Yang S et al. Green tea polyphenols reverse cooperation between c-Rel and CK2 that induces the aryl hydrocarbon receptor, slug, and an invasive phenotype. *Cancer Res* 2007 December 15;67(24):11742-50.

(54) MacPherson MR, Molina P, Souchelnytskyi S et al. Phosphorylation of serine 11 and serine 92 as new positive regulators of human Snail1 function: potential involvement of casein kinase-2 and the cAMP-activated kinase protein kinase A. *Mol Biol Cell* 2010 January 15;21(2):244-53.

(55) Su YW, Xie TX, Sano D, Myers JN. IL-6 stabilizes Twist and enhances tumor cell motility in head and neck cancer cells through activation of casein kinase 2. *PLoS ONE* 2011;6(4):e19412.

(56) Zou J, Luo H, Zeng Q, Dong Z, Wu D, Liu L. Protein kinase CK2alpha is overexpressed in colorectal cancer and modulates cell proliferation and invasion via regulating EMT-related genes. *J Transl Med* 2011;9:97.

(57) Kim J, Hwan KS. CK2 Inhibitor CX-4945 Blocks TGF-beta1-Induced Epithelial-to-Mesenchymal Transition in A549 Human Lung Adenocarcinoma Cells. *PLoS ONE* 2013;8(9):e74342.

(58) Deshiere A, Duchemin-Pelletier E, Spreux E et al. Regulation of epithelial to mesenchymal transition: CK2beta on stage. *Mol Cell Biochem* 2011 October;356(1-2):11-20.

(59) Deshiere A, Duchemin-Pelletier E, Spreux E et al. Unbalanced expression of CK2 kinase subunits is sufficient to drive epithelial-to-mesenchymal transition by Snail1 induction. *Oncogene* 2013 March 14;32(11):1373-83.

(60) Siddiqui-Jain A, Drygin D, Streiner N et al. CX-4945, an orally bioavailable selective inhibitor of protein kinase CK2, inhibits prosurvival and angiogenic signaling and exhibits antitumor efficacy. *Cancer Res* 2010 December 15;70(24):10288-98.

(61) Pierre F, Chua PC, O'Brien SE et al. Pre-clinical characterization of CX-4945, a potent and selective small molecule inhibitor of CK2 for the treatment of cancer. *Mol Cell Biochem* 2011 October;356(1-2):37-43.

(62) Siddiqui-Jain A, Bliesath J, Macalino D et al. CK2 inhibitor CX-4945 suppresses DNA repair response triggered by DNA-targeted anticancer drugs and augments efficacy: mechanistic rationale for drug combination therapy. *Mol Cancer Ther* 2012 April;11(4):994-1005.

(63) Song DH, Sussman DJ, Seldin DC. Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. *J Biol Chem* 2000 August 4;275(31):23790-7.

(64) Liu LZ, Fang J, Zhou Q, Hu X, Shi X, Jiang BH. Apigenin inhibits expression of vascular endothelial growth factor and angiogenesis in human lung cancer cells: implication of chemoprevention of lung cancer. *Mol Pharmacol* 2005 September;68(3):635-43.

(65) Shukla S, Mishra A, Fu P, MacLennan GT, Resnick MI, Gupta S. Up-regulation of insulin-like growth factor binding protein-3 by apigenin leads to growth inhibition and apoptosis of 22Rv1 xenograft in athymic nude mice. *FASEB J* 2005 December;19(14):2042-4.

(66) Fang J, Zhou Q, Liu LZ et al. Apigenin inhibits tumor angiogenesis through decreasing HIF-1alpha and VEGF expression. *Carcinogenesis* 2007 April;28(4):858-64.

(67) Hu XW, Meng D, Fang J. Apigenin inhibited migration and invasion of human ovarian cancer A2780 cells through focal adhesion kinase. *Carcinogenesis* 2008 December;29(12):2369-76.

(68) Lamy S, Bedard V, Labbe D et al. The dietary flavones apigenin and luteolin impair smooth muscle cell migration and VEGF expression through inhibition of PDGFR-beta phosphorylation. *Cancer Prev Res (Phila)* 2008 November;1(6):452-9.

(69) King JC, Lu QY, Li G et al. Evidence for activation of mutated p53 by apigenin in human pancreatic cancer. *Biochim Biophys Acta* 2012 February;1823(2):593-604.

(70) Torkin R, Lavoie JF, Kaplan DR, Yeger H. Induction of caspase-dependent, p53-mediated apoptosis by apigenin in human neuroblastoma. *Mol Cancer Ther* 2005 January;4(1):1-11.

(71) Shukla S, Gupta S. Molecular targets for apigenin-induced cell cycle arrest and apoptosis in prostate cancer cell xenograft. *Mol Cancer Ther* 2006 April;5(4):843-52.

(72) Chen D, Landis-Piwowar KR, Chen MS, Dou QP. Inhibition of proteasome activity by the dietary flavonoid apigenin is associated with growth inhibition in cultured breast cancer cells and xenografts. *Breast Cancer Res* 2007;9(6):R80.

(73) Kaur P, Shukla S, Gupta S. Plant flavonoid apigenin inactivates Akt to trigger apoptosis in human prostate cancer: an in vitro and in vivo study. *Carcinogenesis* 2008 November;29(11):2210-7.

(74) Shukla S, Gupta S. Apigenin suppresses insulin-like growth factor I receptor signaling in human prostate cancer: an in vitro and in vivo study. *Mol Carcinog* 2009 March;48(3):243-52.

(75) Cai J, Zhao XL, Liu AW, Nian H, Zhang SH. Apigenin inhibits hepatoma cell growth through alteration of gene expression patterns. *Phytomedicine* 2011 March 15;18(5):366-73.

(76) Wang QR, Yao XQ, Wen G et al. Apigenin suppresses the growth of colorectal cancer xenografts via phosphorylation and up-regulated FADD expression. *Oncol Lett* 2011 January;2(1):43-7.

(77) Pandey M, Kaur P, Shukla S, Abbas A, Fu P, Gupta S. Plant flavone apigenin inhibits HDAC and remodels chromatin to induce growth arrest and apoptosis in human prostate cancer cells: in vitro and in vivo study. *Mol Carcinog* 2012 December;51(12):952-62.

(78) Budhraja A, Gao N, Zhang Z et al. Apigenin induces apoptosis in human leukemia cells and exhibits anti-leukemic activity in vivo. *Mol Cancer Ther* 2012 January;11(1):132-42.

(79) Mafuvadze B, Liang Y, Besch-Williford C, Zhang X, Hyder SM. Apigenin induces apoptosis and blocks growth of medroxyprogesterone acetate-dependent BT-474 xenograft tumors. *Horm Cancer* 2012 August;3(4):160-71.

(80) Gao AM, Ke ZP, Wang JN, Yang JY, Chen SY, Chen H. Apigenin sensitizes doxorubicin-resistant hepatocellular carcinoma BEL-7402/ADM cells to doxorubicin via inhibiting PI3K/Akt/Nrf2 pathway. *Carcinogenesis* 2013 August;34(8):1806-14.

(81) Hussain AR, Khan AS, Ahmed SO et al. Apigenin induces apoptosis via downregulation of S-phase kinase-associated protein 2-mediated induction of p27Kip1 in primary effusion lymphoma cells. *Cell Prolif* 2010 April;43(2):170-83.

(82) He J, Xu Q, Wang M et al. Oral Administration of Apigenin Inhibits Metastasis through AKT/P70S6K1/MMP-9 Pathway in Orthotopic Ovarian Tumor Model. *Int J Mol Sci* 2012;13(6):7271-82.

(83) Tong X, Pelling JC. Targeting the PI3K/Akt/mTOR axis by apigenin for cancer prevention. *Anticancer Agents Med Chem* 2013 September;13(7):971-8.

(84)   Gao AM, Ke ZP, Wang JN, Yang JY, Chen SY, Chen H. Apigenin sensitizes doxorubicin-resistant hepatocellular carcinoma BEL-7402/ADM cells to doxorubicin via inhibiting PI3K/Akt/Nrf2 pathway. *Carcinogenesis* 2013 August;34(8):1806-14.

(85)   Chunhua L, Donglan L, Xiuqiong F et al. Apigenin up-regulates transgelin and inhibits invasion and migration of colorectal cancer through decreased phosphorylation of AKT. *J Nutr Biochem* 2013 October;24(10):1766-75.

(86)   Osada M, Imaoka S, Funae Y. Apigenin suppresses the expression of VEGF, an important factor for angiogenesis, in endothelial cells via degradation of HIF-1alpha protein. *FEBS Lett* 2004 September 24;575(1-3):59-63.

(87)   Mottet D, Ruys SP, Demazy C, Raes M, Michiels C. Role for casein kinase 2 in the regulation of HIF-1 activity. *Int J Cancer* 2005 December 10;117(5):764-74.

(88)   Fang J, Zhou Q, Liu LZ et al. Apigenin inhibits tumor angiogenesis through decreasing HIF-1alpha and VEGF expression. *Carcinogenesis* 2007 April;28(4):858-64.

(89)   Mirzoeva S, Kim ND, Chiu K, Franzen CA, Bergan RC, Pelling JC. Inhibition of HIF-1 alpha and VEGF expression by the chemopreventive bioflavonoid apigenin is accompanied by Akt inhibition in human prostate carcinoma PC3-M cells. *Mol Carcinog* 2008 September;47(9):686-700.

(90)   Melstrom LG, Salabat MR, Ding XZ et al. Apigenin down-regulates the hypoxia response genes: HIF-1alpha, GLUT-1, and VEGF in human pancreatic cancer cells. *J Surg Res* 2011 May 15;167(2):173-81.

(91)   Gupta S, Afaq F, Mukhtar H. Involvement of nuclear factor-kappa B, Bax and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. *Oncogene* 2002 May 23;21(23):3727-38.

(92)   Lee SH, Ryu JK, Lee KY et al. Enhanced anti-tumor effect of combination therapy with gemcitabine and apigenin in pancreatic cancer. *Cancer Lett* 2008 January 18;259(1):39-49.

(93)   Seo HS, Choi HS, Kim SR et al. Apigenin induces apoptosis via extrinsic pathway, inducing p53 and inhibiting STAT3 and NFkappaB signaling in HER2-overexpressing breast cancer cells. *Mol Cell Biochem* 2012 July;366(1-2):319-34.

(94)   Johnson JL, Gonzalez de ME. Interactions between dietary flavonoids apigenin or luteolin and chemotherapeutic drugs to potentiate anti-proliferative effect on human pancreatic cancer cells, in vitro. *Food Chem Toxicol* 2013 October;60:83-91.

(95)   Lamy S, Akla N, Ouanouki A, Lord-Dufour S, Beliveau R. Diet-derived polyphenols inhibit angiogenesis by modulating the interleukin-6/STAT3 pathway. *Exp Cell Res* 2012 August 1;318(13):1586-96.

(96)   Song DH, Sussman DJ, Seldin DC. Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. *J Biol Chem* 2000 August 4;275(31):23790-7.

(97)   Shukla S, MacLennan GT, Flask CA et al. Blockade of beta-catenin signaling by plant flavonoid apigenin suppresses prostate carcinogenesis in TRAMP mice. *Cancer Res* 2007 July 15;67(14):6925-35.

(98)   Tsai CH, Lin FM, Yang YC et al. Herbal extract of Wedelia chinensis attenuates androgen receptor activity and orthotopic growth of prostate cancer in nude mice. *Clin Cancer Res* 2009 September 1;15(17):5435-44.

(99)   Lin FM, Chen LR, Lin EH et al. Compounds from Wedelia chinensis synergistically suppress androgen activity and growth in prostate cancer cells. *Carcinogenesis* 2007 December;28(12):2521-9.

(100)   Cai X, Liu X. Inhibition of Thr-55 phosphorylation restores p53 nuclear localization and sensitizes cancer cells to DNA damage. *Proc Natl Acad Sci U S A* 2008 November 4;105(44):16958-63.

(101)   Zhong Y, Krisanapun C, Lee SH et al. Molecular targets of apigenin in colorectal cancer cells: involvement of p21, NAG-1 and p53. *Eur J Cancer* 2010 December;46(18):3365-74.

(102)   Dixit D, Sharma V, Ghosh S, Mehta VS, Sen E. Inhibition of Casein kinase-2 induces p53-dependent cell cycle arrest and sensitizes glioblastoma cells to tumor necrosis factor (TNFalpha)-induced apoptosis through SIRT1 inhibition. *Cell Death Dis* 2012;3:e271.

(103)   Choi EJ, Kim GH. Apigenin causes G(2)/M arrest associated with the modulation of p21(Cip1) and Cdc2 and activates p53-dependent apoptosis pathway in human breast cancer SK-BR-3 cells. *J Nutr Biochem* 2009 April;20(4):285-90.

(104)   Shukla S, Gupta S. Apigenin-induced prostate cancer cell death is initiated by reactive oxygen species and p53 activation. *Free Radic Biol Med* 2008 May 15;44(10):1833-45.

(105)   Chiang LC, Ng LT, Lin IC, Kuo PL, Lin CC. Anti-proliferative effect of apigenin and its apoptotic induction in human Hep G2 cells. *Cancer Lett* 2006 June 18;237(2):207-14.

(106)   Zheng PW, Chiang LC, Lin CC. Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. *Life Sci* 2005 February 4;76(12):1367-79.

(107)   Takagaki N, Sowa Y, Oki T, Nakanishi R, Yogosawa S, Sakai T. Apigenin induces cell cycle arrest and p21/WAF1 expression in a p53-independent pathway. *Int J Oncol* 2005 January;26(1):185-9.

(108)   Lolli G, Cozza G, Mazzorana M et al. Inhibition of protein kinase CK2 by flavonoids and tyrphostins. A structural insight. *Biochemistry* 2012 August 7;51(31):6097-107.

(109)   Attoub S, Hassan AH, Vanhoecke B et al. Inhibition of cell survival, invasion, tumor growth and histone deacetylase activity by the dietary flavonoid luteolin in human epithelioid cancer cells. *Eur J Pharmacol* 2011 January 25;651(1-3):18-25.

(110)   Yang SF, Yang WE, Chang HR, Chu SC, Hsieh YS. Luteolin induces apoptosis in oral squamous cancer cells. *J Dent Res* 2008 April;87(4):401-6.

(111) Chiu FL, Lin JK. Downregulation of androgen receptor expression by luteolin causes inhibition of cell proliferation and induction of apoptosis in human prostate cancer cells and xenografts. *Prostate* 2008 January 1;68(1):61-71.

(112) Bagli E, Stefaniotou M, Morbidelli L et al. Luteolin inhibits vascular endothelial growth factor-induced angiogenesis; inhibition of endothelial cell survival and proliferation by targeting phosphatidylinositol 3'-kinase activity. *Cancer Res* 2004 November 1;64(21):7936-46.

(113) Selvendiran K, Koga H, Ueno T et al. Luteolin promotes degradation in signal transducer and activator of transcription 3 in human hepatoma cells: an implication for the antitumor potential of flavonoids. *Cancer Res* 2006 May 1;66(9):4826-34.

(114) Shi R, Huang Q, Zhu X et al. Luteolin sensitizes the anticancer effect of cisplatin via c-Jun NH2-terminal kinase-mediated p53 phosphorylation and stabilization. *Mol Cancer Ther* 2007 April;6(4):1338-47.

(115) Zhou Q, Yan B, Hu X, Li XB, Zhang J, Fang J. Luteolin inhibits invasion of prostate cancer PC3 cells through E-cadherin. *Mol Cancer Ther* 2009 June;8(6):1684-91.

(116) Hwang JT, Park OJ, Lee YK et al. Anti-tumor effect of luteolin is accompanied by AMP-activated protein kinase and nuclear factor-kappaB modulation in HepG2 hepatocarcinoma cells. *Int J Mol Med* 2011 July;28(1):25-31.

(117) Tsai CH, Lin FM, Yang YC et al. Herbal extract of Wedelia chinensis attenuates androgen receptor activity and orthotopic growth of prostate cancer in nude mice. *Clin Cancer Res* 2009 September 1;15(17):5435-44.

(118) Yan J, Wang Q, Zheng X et al. Luteolin enhances TNF-related apoptosis-inducing ligand's anticancer activity in a lung cancer xenograft mouse model. *Biochem Biophys Res Commun* 2012 January 13;417(2):842-6.

(119) Lee EJ, Oh SY, Sung MK. Luteolin exerts anti-tumor activity through the suppression of epidermal growth factor receptor-mediated pathway in MDA-MB-231 ER-negative breast cancer cells. *Food Chem Toxicol* 2012 November;50(11):4136-43.

(120) Pratheeshkumar P, Son YO, Budhraja A et al. Luteolin inhibits human prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis. *PLoS ONE* 2012;7(12):e52279.

(121) Huang WW, Chiu YJ, Fan MJ et al. Kaempferol induced apoptosis via endoplasmic reticulum stress and mitochondria-dependent pathway in human osteosarcoma U-2 OS cells. *Mol Nutr Food Res* 2010 November;54(11):1585-95.

(122) Khan N, Asim M, Afaq F, Abu ZM, Mukhtar H. A novel dietary flavonoid fisetin inhibits androgen receptor signaling and tumor growth in athymic nude mice. *Cancer Res* 2008 October 15;68(20):8555-63.

(123) Tripathi R, Samadder T, Gupta S, Surolia A, Shaha C. Anticancer activity of a combination of cisplatin and fisetin in embryonal carcinoma cells and xenograft tumors. *Mol Cancer Ther* 2011 February;10(2):255-68.

(124) Ying TH, Yang SF, Tsai SJ et al. Fisetin induces apoptosis in human cervical cancer HeLa cells through ERK1/2-mediated activation of caspase-8-/caspase-3-dependent pathway. *Arch Toxicol* 2012 February;86(2):263-73.

(125) Zhou W, Kallifatidis G, Baumann B et al. Dietary polyphenol quercetin targets pancreatic cancer stem cells. *Int J Oncol* 2010 September;37(3):551-61.

(126) Zhong X, Wu K, He S, Ma S, Kong L. [Effects of quercetin on the proliferation and apoptosis in transplantation tumor of breast cancer in nude mice]. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2003 July;34(3):439-42.

(127) Ma ZS, Huynh TH, Ng CP, Do PT, Nguyen TH, Huynh H. Reduction of CWR22 prostate tumor xenograft growth by combined tamoxifen-quercetin treatment is associated with inhibition of angiogenesis and cellular proliferation. *Int J Oncol* 2004 May;24(5):1297-304.

(128) Dechsupa S, Kothan S, Vergote J et al. Quercetin, Siamois 1 and Siamois 2 induce apoptosis in human breast cancer MDA-mB-435 cells xenograft in vivo. *Cancer Biol Ther* 2007 January;6(1):56-61.

(129) Sun ZJ, Chen G, Hu X et al. Activation of PI3K/Akt/IKK-alpha/NF-kappaB signaling pathway is required for the apoptosis-evasion in human salivary adenoid cystic carcinoma: its inhibition by quercetin. *Apoptosis* 2010 July;15(7):850-63.

(130) Cheng S, Gao N, Zhang Z et al. Quercetin induces tumor-selective apoptosis through downregulation of Mcl-1 and activation of Bax. *Clin Cancer Res* 2010 December 1;16(23):5679-91.

(131) Long Q, Xiel Y, Huang Y et al. Induction of apoptosis and inhibition of angiogenesis by PEGylated liposomal quercetin in both cisplatin-sensitive and cisplatin-resistant ovarian cancers. *J Biomed Nanotechnol* 2013 June;9(6):965-75.

(132) Huang CY, Chan CY, Chou IT, Lien CH, Hung HC, Lee MF. Quercetin induces growth arrest through activation of FOXO1 transcription factor in EGFR-overexpressing oral cancer cells. *J Nutr Biochem* 2013 September;24(9):1596-603.

(133) Chan ST, Yang NC, Huang CS, Liao JW, Yeh SL. Quercetin enhances the antitumor activity of trichostatin A through upregulation of p53 protein expression in vitro and in vivo. *PLoS ONE* 2013;8(1):e54255.

(134) Wang G, Zhang J, Liu L, Sharma S, Dong Q. Quercetin potentiates doxorubicin mediated antitumor effects against liver cancer through p53/Bcl-xl. *PLoS ONE* 2012;7(12):e51764.

(135) Pratheeshkumar P, Budhraja A, Son YO et al. Quercetin inhibits angiogenesis mediated human prostate tumor growth by targeting V. *PLoS ONE* 2012;7(10):e47516.

(136) Gao X, Wang B, Wei X et al. Anticancer effect and mechanism of polymer micelle-encapsulated quercetin on ovarian cancer. *Nanoscale* 2012 November 21;4(22):7021-30.

(137) Angst E, Park JL, Moro A et al. The flavonoid quercetin inhibits pancreatic cancer growth in vitro and in vivo. *Pancreas* 2013 March;42(2):223-9.

(138) Kim HS, Wannatung T, Lee S et al. Quercetin enhances hypoxia-mediated apoptosis via direct inhibition of AMPK activity in HCT116 colon cancer. *Apoptosis* 2012 September;17(9):938-49.

(139) Tan BJ, Liu Y, Chang KL, Lim BK, Chiu GN. Perorally active nanomicellar formulation of quercetin in the treatment of lung cancer. *Int J Nanomedicine* 2012;7:651-61.

(140) Zheng SY, Li Y, Jiang D, Zhao J, Ge JF. Anticancer effect and apoptosis induction by quercetin in the human lung cancer cell line A-549. *Mol Med Rep* 2012 March;5(3):822-6.

(141) Lin C, Yu Y, Zhao HG, Yang A, Yan H, Cui Y. Combination of quercetin with radiotherapy enhances tumor radiosensitivity in vitro and in vivo. *Radiother Oncol* 2012 September;104(3):395-400.

(142) Wang K, Liu R, Li J et al. Quercetin induces protective autophagy in gastric cancer cells: involvement of Akt-mTOR- and hypoxia-induced factor 1alpha-mediated signaling. *Autophagy* 2011 September;7(9):966-78.

(143) Wong MY, Chiu GN. Liposome formulation of co-encapsulated vincristine and quercetin enhanced antitumor activity in a trastuzumab-insensitive breast tumor xenograft model. *Nanomedicine* 2011 December;7(6):834-40.

(144) Sun F, Zheng XY, Ye J, Wu TT, Wang J, Chen W. Potential anticancer activity of myricetin in human T24 bladder cancer cells both in vitro and in vivo. *Nutr Cancer* 2012;64(4):599-606.

(145) Shia CS, Tsai SY, Kuo SC, Hou YC, Chao PD. Metabolism and pharmacokinetics of 3,3',4',7-tetrahydroxyflavone (fisetin), 5-hydroxyflavone, and 7-hydroxyflavone and antihemolysis effects of fisetin and its serum metabolites. *J Agric Food Chem* 2009 January 14;57(1):83-9.

(146) FISHMAN WH, ANLYAN AJ. The presence of high beta-glucuronidase activity in cancer tissue. *J Biol Chem* 1947 July;169(2):449.

(147) Bosslet K, Straub R, Blumrich M et al. Elucidation of the mechanism enabling tumor selective prodrug monotherapy. *Cancer Res* 1998 March 15;58(6):1195-201.

(148) Paigen K. Mammalian beta-glucuronidase: genetics, molecular biology, and cell biology. *Prog Nucleic Acid Res Mol Biol* 1989;37:155-205.

(149) Estrella V, Chen T, Lloyd M et al. Acidity generated by the tumor microenvironment drives local invasion. *Cancer Res* 2013 March 1;73(5):1524-35.

(150) Murdter TE, Friedel G, Backman JT et al. Dose optimization of a doxorubicin prodrug (HMR 1826) in isolated perfused human lungs: low tumor pH promotes prodrug activation by beta-glucuronidase. *J Pharmacol Exp Ther* 2002 April;301(1):223-8.

(151) Sperker B, Werner U, Murdter TE et al. Expression and function of beta-glucuronidase in pancreatic cancer: potential role in drug targeting. *Naunyn Schmiedebergs Arch Pharmacol* 2000 August;362(2):110-5.

(152) de GM, Boven E, Scheeren HW, Haisma HJ, Pinedo HM. Beta-glucuronidase-mediated drug release. *Curr Pharm Des* 2002;8(15):1391-403.

(153)   Yuan L, Wagatsuma C, Yoshida M et al. Inhibition of human breast cancer growth by GCP (genistein combined polysaccharide) in xenogeneic athymic mice: involvement of genistein biotransformation by beta-glucuronidase from tumor tissues. *Mutat Res* 2003 February;523-524:55-62.

(154)   Chen X, Wu B, Wang PG. Glucuronides in anti-cancer therapy. *Curr Med Chem Anticancer Agents* 2003 March;3(2):139-50.

(155)   Oi N, Hashimoto T, Kanazawa K. Metabolic conversion of dietary quercetin from its conjugate to active aglycone following the induction of hepatocarcinogenesis in fisher 344 rats. *J Agric Food Chem* 2008 January 23;56(2):577-83.

(156)   Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. *Cancer Lett* 2008 October 8;269(2):315-25.

(157)   Legigan T, Clarhaut J, Renoux B et al. Synthesis and antitumor efficacy of a beta-glucuronidase-responsive albumin-binding prodrug of doxorubicin. *J Med Chem* 2012 May 10;55(9):4516-20.

(158)   Chen KC, Schmuck K, Tietze LF, Roffler SR. Selective cancer therapy by extracellular activation of a highly potent glycosidic duocarmycin analogue. *Mol Pharm* 2013 May 6;10(5):1773-82.