Review on Molecular and Chemopreventive Potential of Nimbolide in Cancer

Perumal Elumalai*, Jagadeesan Arunakaran

Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai 600113, India

Cancer is the most dreaded disease in human and also major health problem worldwide. Despite its high occurrence, the exact molecular mechanisms of the development and progression are not fully understood. The existing cancer therapy based on allopathic medicine is expensive, exhibits side effects; and may also alter the normal functioning of genes. Thus, a non-toxic and effective mode of treatment is needed to control cancer development and progression. Some medicinal plants offer a safe, effective and affordable remedy to control the cancer progression. Nimbolide, a limnoid derived from the neem (*Azadirachta indica*) leaves and flowers of neem, is widely used in traditional medical practices for treating various human diseases. Nimbolide exhibits several pharmacological effects among which its anticancer activity is the most promising. The previous studies carried out over the decades have shown that nimbolide inhibits cell proliferation and metastasis of cancer cells. This review highlights the current knowledge on the molecular targets that contribute to the observed anticancer activity of nimbolide related to induction of apoptosis and cell cycle arrest; and inhibition of signaling pathways related to cancer progression.

Keywords: apoptosis, cell proliferation, chemoprevention, neoplasm metastasis, nimbolide

Introduction

Cancer is the most dangerous disease of human and a major health problem worldwide [1]. Preclinical studies have shown that cancer is a large group of diseases characterized by the uncontrolled cell growth and spread of abnormal cells. Over the past quarter of a century, an outstanding progress in understanding of the proteins involved in cancer progression has grown, providing chances for identifying new targets for anti-cancer therapy [2, 3]. Present modes of treatment based on synthetic drugs have limited potential, because they are toxic and expensive and also alter cell signaling pathways. Natural drugs that are safe, affordable, and effective are needed to control cancer development and progression. Natural products have been used for thousands of years in the management of several diseases including various types of cancer [4]. Nimbolide was first derived from the leaves and flowers of neem. Neem (*Azadirachta indica*), is a traditional medicinal plant of the Meliaceae family widely distributed in Asia, Africa and other tropical parts of the world. All parts of the neem tree offer amazing potential for medicinal, agricultural and industrial exploitation and have been evaluated for antiinflammatory, antipyretic, antihistamine, antifungal, antitubercular, antiprotozoal, vasodilatory, antiinflammatory, diuretic, spermicidal, antiarthritic, insect repellent, antifeedant, and antiendocrine activities [5].

Studies of extracts from all major parts of neem plant including the leaves, flowers, fruits, and seeds, have shown promising chemopreventive and therapeutic effects in pre-clinical research [6]. Extracts of neem leaf have been reported to be non-toxic and non-mutagenic and are found to possess immunomodulatory as well as anti-inflammatory and anticarcinogenic properties [7]. There are many studies showing the ethanolic extract of neem leaves to possess anticancer activity. Ethanolic neem leaf extract (ENLE) exhibited anticancer activity against N-methyl-N-nitro-N-nitrosoguanidine-induced oxidative stress and gastric carcinogenesis [8]. ENLE induces apoptosis in a prostate cancer cell line (PC-3) by up-regulating the pro-apoptotic
protein Bax and decreasing the level of Bcl-2 protein resulting in DNA fragmentation in prostate cancer cells [9, 10]. Many bioactive compounds are isolated from this plant among which, nimbolide belongs to the limonoid group. It is the major component of the leaves of A. indica.

Nimbolide (5,7,4′-trihydroxy-3′,5′-diprenylflavanone), is a tetrannortriterpenoid with α, β-unsaturated system and δ-lactonic ring (Fig. 1) [11]. It has been shown to exhibit numerous types of biological activities, including, antimalarial [12], anti-bacterial activity [13], anti-feedent, [14] and anticancer activities [15-19]. Literature evidence reveals that α, β-unsaturated ketone structural element is responsible for the anticancer activity of nimbolide [20, 21].

Mechanism of Action of Nimbolide in Cancer Prevention

Cancers are abnormal cell growth caused by genomic modification. So, any agent that has anti-cancer activity either protects genetic material from alterations or kills the genetically altered cancer cells. The active component nimbolide from neem acts on cancer cells and kill them by altering the several molecular pathways. The cytotoxicity of nimbolide has been widely studied over the last several years in a large variety of cancer cell lines [22]. It was reported recently that nimbolide inhibited cancer progression by influencing multiple mechanisms, including prevention of procarcinogen activation and oxidative DNA damage, up-regulation of antioxidant and induction of apoptosis, inhibition of tumor cell proliferation, invasion, angiogenesis, and metastasis [19, 23-26]. The potential mechanisms of cancer prevention by nimbolide are described below and summarized in Fig. 2.

Inhibition of Cancer Cell Proliferation and Growth

Tumorigenesis and cancer progression are thought to be the result of some changes in different types of genetic pathways [27, 28]. Nimbolide, a chief constituent of neem, shows a vital role in cancer prevention and treatment through the modulation of various biological activities, including molecular cascades. However, understanding the mechanism of action of nimbolide in the activation or inactivation of genetic pathways will provide significant information to develop therapeutic approaches to manage various types of cancers.

Nimbolide induces in vitro cytotoxic activity against human cancer cell lines [20]. Nimbolide decreased cell viability, with an IC50 ranging from 4 to 10 μM and averaging

---

Fig. 1. Structure of nimbolide. Molecular formula, C27H30O7; molecular weight, 466.5; appearance, white to off-white solid.

Fig. 2. Potential mechanisms of cancer prevention by nimbolide.
Fig. 3. Detailed scheme of the growth factor signalling pathways targeted by nimbolide in cancer cells. Nimbolide strongly inhibits IGF-IR and also affects downstream signalling via the MAPK (ERK) and PI3K/Akt. Transcription factors like NF-κB, c-Myc, and β-catenin consequently cannot be activated, thus modulating target gene expression. As most of the target genes are implicated in cell cycle regulation and proliferation, the cancer cells are finally blocked in cell cycle progression. ERK, extracellular signal-regulated kinase; IGF-IR, insulin-like growth factor 1 receptor; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase tensin homolog deleted on chromosome 10.
significantly decreased in nimbolide-treated cells. This is further supported by a significant increase in the protein expression of PTEN upon nimbolide treatment in breast cancer cell lines (Fig. 3). Thereby, nimbolide inhibits the cell survival and proliferation of breast cancer and prostate cancer cells [19, 43].

The Effects of Nimbolide on NF-κB Signaling

NF-κB comprises a family of transcription factors involved in the regulation of a wide variety of biological responses. NF-κB has a well-known function in the regulation of immune responses and inflammation, but growing evidence supports a major role in oncogenesis. NF-κB regulates the expression of genes involved in many processes that play a key role in the development and progression of cancer, such as proliferation, migration, and apoptosis [44, 45]. This transcription factor is localized in the cytosol and is blocked by Iκb. Activation of NF-κB may result from different signaling pathways triggered by a variety of cytokines, growth factors, and tyrosine kinases [44].

Recently, it was studied that nimbolide exerts potent anticancer effects in HepG2 cells by inhibiting NF-κB activation and its downstream events, such as activation of the Wnt/β-catenin pathway and apoptosis evasion. Inhibitors of NF-κB which can block several signalling pathway, have developed as a successful candidates for novel anti-cancer regimens. Thus, nimbolide, by targeting multiple components of the NF-κB signaling pathway to inhibit tumor progression, is a promising agent for cancer prevention and therapy [17]. Gupta et al. [16] found that suppression of NF-κB activation by nimbolide was caused by inhibition of IKK, which led to suppression of IκB phosphorylation and degradation, nuclear translocation, DNA binding, and gene transcription in myeloid and leukemic cells. Nimbolide significantly decreased the protein expression of IKKα, IKKβ, and NF-κB in breast cancer cell lines [46].

Nimbolide Inhibits Cancer Cell Metastasis

Metastasis is the process by which a cancer cell leaves the primary tumor, travels to a distant site via the circulatory system, and begins a secondary tumor. In order to metastasize, cancer cells must invade through the basement membrane and the extracellular matrix (ECM). Proteolysis of the ECM is an important step in metastasis, and the process is associated with the upregulated production and activity of several ECM-degrading proteases. The matrix metalloproteinases (MMPs) constitute a family of structurally related, zinc-dependent endopeptidases that are capable of degrading the protein components of the ECM and basement membrane and release growth factors from ECM stores [47, 48]. MMP activity is regulated by specific inhibitors, the tissue inhibitors of MMP (TIMPs). Activation of chemokines and the urokinase plasminogen activator (uPA)/uPA receptor (uPAR) system is also a central mediator of tumor-cell migration and invasion, and IGF-I signaling can influence the uPAR pathway.

Chemokines are chemotactic cytokines that cause the directed migration of leukocytes and are induced by inflammatory cytokines, growth factors, and pathogenic stimuli. Chemokine signaling results in the transcription of target genes that are involved in cell invasion, motility, and survival. The upregulation of chemokine molecules in tumor biology as compared with “normal” cells confers chemokines as “a magic bullet,” as targeting them can potentially hit cancer cells and their metastasis, leaving non-affected cells unharmed [49]. The expression of the chemokines CCL2, CXCL12, and CXCL8 and their receptors CCR2, CXCR1, CXCR2, and CXCR4 is significantly decreased in nimbolide-treated breast cancer cell lines [46].

uPA is a serine protease that is involved in cancer progression, mainly invasion and metastasis [50]. uPA can be observed as a multifunctional protein that is involved in both proteolysis and signal transduction [51]. Recently, nimbolide has been shown to interfere with the expression of NF-κB-regulated proteins, like Bcl-2, cyclooxygenase 2, MMP-9, and VEGF, by inhibiting IKK. Therefore, it is likely that nimbolide will interfere with cell migration invasion and angiogenesis [29]. The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by prevention of procarcinogen activation and oxidative DNA damage, upregulation of antioxidants and carcinoen detoxification enzymes, and inhibition of tumor invasion and angiogenesis [24]. The protein expression of MMP-2 and -9 is significantly decreased, and TIMP-2 expression is increased in nimbolide-treated breast cancer cells [46]. Other studies also reported that the protein expression and activity of MMP-2 and -9 were significantly decreased in myeloid leukemia cell lines [16] and colon cancer cells [29]. Limonoid-treated xenografts exhibited significant down-regulation in the expression of proteins involved in tumor cell survival (Bcl-2, Bcl-xl, c-IAP-1, survivin, and Mcl-1), proliferation (c-Myc and cyclin D1), invasion (MMP-9, intercellular adhesion molecule 1), metastasis (CXCR4), and angiogenesis (VEGF) [26]. The migration and invasive potential of the MCF-7 and MDA-MB-231 cell lines were considerably suppressed upon nimbolide treatment. The results showed that nimbolide downregulated the expression of uPA, uPAR, chemokines, phospho-epidermal growth
factor receptor, vascular endothelial growth factor, NF-κB, IKKα, IKKβ, MMP-2, and MMP-9 and upregulated the expression of TIMP-2, suggesting that nimbolide inhibits angiogenesis and metastasis of breast cancer [46].

Inhibition of Cell Cycle and Induction of Apoptosis by Nimbolide

The loss of the ability to regulate the cell-cycle is characteristic of cancer cells and results in uncontrollable proliferation. Processing cells through the first gap (G) phase of the cell cycle is a step that is frequently disordered in cancer [52]. Nimbolide has been investigated in different studies for its ability to mediate cell cycle arrest. In many *in vitro* and *in vivo* studies, nimbolide has shown cell cycle-regulatory effects [18, 25, 53, 54]. It has been reported that nimbolide inhibits cell proliferation by interfering with cell cycle kinetics by inducing G0/G1 and S phase arrest, primarily caused through the repression of cyclin A/cyclin D1 [16, 54, 55].

In another interesting molecular study, a Japanese group has proved that nimbolide, a triterpenoid present in certain edible parts of *A. indica*, arrested HT-29 cells in the G2/M and G0/G1 stages apparently through upregulation of p21, which is a well-known downstream effector of the p53. p53 is a very important anticancer protein that regulates a large number of genes that are involved in cancer progression. Nimbolide has also been shown to upregulate cyclin D2 and CDK2 and to suppress the expression of cyclin A, cyclin E, CDK2, and Rad17 at the same time [53]. Flow cytometric analysis of U937 cells showed that nimbolide treatment (1–2.5 μM) resulted in cell cycle disruption by decreasing the number of cells in G0/G1 phase, with initial increases in S and G2/M phases. It is shown that nimbolide can affect cell cycle progression and induce apoptosis in colon cancer, oral carcinoma, and cervical cancer [18, 25, 55]. Nimbolide significantly suppressed the viability of HeLa cells in a dose-dependent manner by inducing cell cycle arrest at G0/G1 phase, accompanied by p21 accumulation and downregulation of the cell cycle regulatory proteins cyclin B, cyclin D1, and proliferating cell nuclear antigen (PCNA) [55].

Nimbolide treatment results in the accumulation of cells in G0/G1 phase and decreased in S-phase by up regulating p21 and downregulating the cell cycle-regulatory proteins cyclins and PCNA. Cyclin D1 is known as a proto-oncogene whose gene amplification and protein overexpression of which are frequently observed in tumor cells. The activated cyclin D1/CDK4 and cyclin D1/CDK6 complex phosphorylates the retinoblastoma protein to induce the expression of target genes essential for S phase entry, facilitating the progression from G1 to S phase [56]. Cyclin B1 is a G2/mitotic-specific protein that plays a role in the initiation of mitosis and tumorigenesis [57]. It was reported that cyclin B1 depletion inhibits proliferation and induces apoptosis in human tumor cells [57]. Nimbolide treatment decreases cyclin (A1, B1, C, D1, and E1) expression in breast cancer cells. p21\textsuperscript{N16K\textsuperscript{C14S}}, originally identified as an inhibitor of the cyclin/CDK complexes, has also been shown to have a role as an adaptor protein that assembles and promotes the kinase activity of cyclin D/CDK4 complexes [58]. The level of p21 was significantly increased in nimbolide-treated breast cancer cell lines. PCNA, a cofactor for DNA polymerase δ, plays a central role in cell cycle progression [59]. PCNA is involved in a wide range of cellular functions, including DNA replication, repair, and epigenetic maintenance, and is often used as a diagnostic and prognostic marker. The protein expression of PCNA is decreased in nimbolide-treated breast cancer cells [19]. Nimbolide directly inhibited CDK4/CDK6 kinase activity, leading to hypophosphorylation of the retinoblastoma protein, cell cycle arrest at G1-S, and cell death [42]. In animal tumor models, nimbolide (100 μg/kg) has been shown to exhibit chemopreventive activity against 7,12-dimethylbenzanthracene (DMBA) 3-induced hamster buccal pouch carcinogenesis by downregulating proteins involved in cell cycle progression and transduce apoptosis by both the intrinsic and extrinsic pathways [25].

Apoptosis, or programmed cell death, is essential for the maintenance of development and homeostasis of multicellular organisms by eliminating superfluous or unwanted cells. Any alteration or change in the normal process of apoptosis may increase cell survival and support tumor development and progression [60]. The extrinsic and intrinsic pathways represent the two major well-studied apoptotic processes. Inefficient apoptosis is considered one of the hallmarks of tumorigenicity [61]. Moreover, induction of apoptosis is an important target for cancer therapy [62]. The extrinsic pathway is initiated by cell surface-expressed death receptors of the tumor necrosis factor superfamily. One of the central pathways of apoptosis is initiated by cytokines, such as tumor necrosis factor-α, Fas ligand (FasL), and tumor necrosis factor-α-related apoptosis-inducing ligand (TRAIL) [63]. The intrinsic pathway is initiated by anticancer drugs, growth factor withdrawal, or hypoxia or via induction of oncopgenes. These stimuli induce permeabilization of the outer mitochondrial membrane and activate the mitochondrial pathway [64].

Caspases are a family of evolutionarily conserved cysteine proteases that play a essential role in the majority of apoptotic pathways. Death signals activate the proteolytic cascade of caspases through two main pathways: an extrinsic and intrinsic pathway [65]. Both pathways converge to the activation of caspase-3, the closer homolog of Caenor-
Fig. 4. Nimbolide shows an important role in cancer prevention via inhibition of cell survival, cell cycle progression, invasion, migration and induction of apoptosis. The diverse molecular targets influenced by nimbolide include the growth factors and their receptor, signaling molecules, transcription factors, protein kinases, enzymes and genes regulating cell proliferation and apoptosis. Red down arrow indicates that these molecules are down-regulated by nimbolide, whereas, green up arrow indicate that these molecules are up-regulated by nimbolide. Bad, Bcl-2-associated death promoter; Bax, Bcl-2-associated X protein; Bcl-2, B cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra-large; DR-5, death receptor-5; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; FADD, Fas-associated death domain; FasL, Fatty acid synthase ligand; ICAM, Intercellular adhesion molecule; IGF-1, insulin-like growth factor 1; IGF-binding protein 3; IKB, IκB kinase; IRS, insulin receptor substrate; MCL-1, myeloid cell leukemia 1; MMPs, matrix metalloproteinases; NF-κB, nuclear factor kappa B; PARP, poly(ADP) ribose polymerase; PCNA, proliferating cell nuclear antigen; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase tensin homolog deleted on chromosome 10; Rb, retinoblastoma; TIMP, tissue inhibitor of matrix metalloproteinase; TRAIL, tumor necrosis factor apoptosis inducing ligand; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; XIAP, X-linked inhibitor of apoptosis protein; uPA, urokinase plasminogen antigen; uPAR, urokinase plasminogen antigen receptor.
upregulating pro-apoptotic protein (Bad, Bax, FasL, FADD, TRAIL, and cytochrome c) expression and down-regulating anti-apoptotic proteins (Bcl-2, Bcl-xl, Mcl-1, and XIAP-1). Nimbolede activates caspase-3, -8, and -9, which favors the cleavage of poly(ADP) ribose polymerase into 115- and 85-kDa peptides, thus inducing apoptosis in breast cancer cell lines. Further, the nimbolede-induced apoptotic cells were detected using DAPI and AO/EtBr dual staining. After treatment with nimbolede, the cells exhibited the typical morphological changes associated with apoptosis: cell shrinkage, nuclear condensation, and membrane blebbing [30]. Activation of caspase-3, -8, and -9, suggests that nimbolede potentiated both the extrinsic and intrinsic pathways of apoptosis in human breast and colon cancer cells [68, 30]. The overview of all of the signaling molecules modulated by nimbolede is shown in Fig. 4.

**Conclusion and Future Perspectives**

Cancer has continually been the leading cause of death worldwide for decades. Thus, researchers have actively devoted themselves to studying cancer therapeutics. The present mode of treatment based on chemotherapy and radiotherapy is very expensive and also exhibits serious side effects in human beings. Keeping in view the significance of herbs, this review is written to show the role of nimbolede in the prevention of various types of cancer through the activation or inactivation of various signaling pathways. These reported features, combined with the absence of side effects and being inexpensive and easy to access, neem and its constituent nimbolede may be proved very effective therapeutics in the management of cancers. Nimbolede is a potential chemopreventive agent that is able to suppress multiple signaling pathways involved in carcinogenesis and hence is an attractive candidate for further research. Currently, nimbolede is undergoing extensive research to determine the dose efficacy and administration form that could enhance its bioavailability and chemopreventive properties. How nimbolede mediates all of these effects is not completely understood. However, most of the anticancer activities assigned to nimbolede have been based on in vitro studies. While in vitro studies predominantly established the anti-cancer effects of nimbolede on cancer cells, its efficiency in vivo has still to be proven.

**Acknowledgments**

The financial support from the Council for Scientific and Industrial Research (India) in the form of a Senior Research Fellowship (CSIR-SRF) to P. Elumalai is greatly acknowledged.
17. Kavitha K, Vidya Priyadarsini R, Anitha P, Ramalingam K, Saktihivel R, Purushothaman G, et al. Nimbolide, a neem limonoid abrogates canonical NF-kappaB and Wnt signaling to induce caspase-dependent apoptosis in human hepatocarcinoma (HepG2) cells. Eur J Pharmacol 2012;681:6-14.

18. Roy MK, Kobori M, Takenaka M, Nakahara K, Shimoto H, Isobe S, et al. Antiproliferative effect on human cancer cell lines after treatment with nimbolide extracted from an edible part of the neem tree (Azadirachta indica). Phytother Res 2007;21:245-250.

19. Elumalai P, Arunkumar R, Benson CS, Sharmila G, Arunakaran J. Nimbolide inhibits IGF-I-mediated PI3K/Akt and MAPK signalling in human breast cancer cell lines (MCF-7 and MDA-MB-231). Cell Biochem Funct 2014;32:476-484.

20. Kigodi PG, Blaskó G, Thebtaranonth Y, Pezzuto JM, Cordell GA. Spectroscopic and biological investigation of nimbolide and 2-deoxonimbolide from Azadirachta indica. J Nat Prod 1989;52:1246-1251.

21. Sastry BS, Suresh Babu K, Hari Babu T, Chandrasekhar S, Sakthivel R, Purushothaman G, et al. Synthesis and biological activity of amide derivatives of nimbolide. Bioorg Med Chem Lett 2006;16:4391-4394.

22. Bodduluru LN, Kasala ER, Thota N, Barua CC, Sistla R. Chemopreventive and therapeutic effects of nimbolide in cancer: the underlying mechanisms. Toxicol In Vitro 2014;28:1026-1035.

23. Priyadarshini RV, Manikandan P, Kumar GH, Nagini S. The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by modulating xenobiotic-metabolizing enzymes, DNA damage, antioxidants, invasion and angiogenesis. Free Radic Res 2009;43:492-504.

24. Harish Kumar G, Vidya Priyadarsini R, Vinothini G, Vidjaya Letchumy P, Nagini S. The neem limonoids azadirachtin and nimbolide inhibit cell proliferation and induce apoptosis in an animal model of oral oncogenesis. Invest New Drugs 2010;28:392-401.

25. Srinivas PV, Saxena AK, et al. Quinone reductase inductors in Azadirachta indica Juss flowers, and their mechanisms of action. Asian Pac J Cancer Prev 2005;6:263-269.

26. Gupta SC, Prasad S, Sethumadhavan DR, Nair MS, Mo YY, Aggarwal BB. Nimbolide, a limonoid triterpene, inhibits growth of human colorectal cancer xenografts by suppressing the proinflammatory microenvironment. Clin Cancer Res 2013;19:4465-4476.

27. Rahman A, Alzohairy M, Mardal AK, Rizvi MA. Expressional evaluation of androgen receptor in transitional cell carcinoma of urinary bladder patients. Br J Med Res 2011;1:223-238.

28. Babiker AM, Rahman AH, Abdalaziz MS, Albatt A, Aly SM, Ahmed HG. Expressional analysis of p16 and cytookeratin19 protein in the genesis of oral squamous cell carcinoma patients. Int J Clin Exp Med 2014;7:1524-1530.

29. Babykutty S, S PE J NR, Kumar MA, Nair MS, Srinivas P, et al. Nimbolide retards tumor cell migration, invasion, and angiogenesis by downregulating MMP-2/9 expression via inhibiting ERK1/2 and reducing DNA-binding activity of NF-kappaB in colon cancer cells. Mol Carcinog 2012;51:475-490.

30. Elumalai P, Gunadharini DN, Senthilkumar K, Banudevi S, Arunakaran R, Benson CS, et al. Induction of apoptosis in human breast cancer cells by nimbolide through extrinsic and intrinsic pathway. Toxicol Lett 2012;215:131-142.

31. Nielsen TO, Andrews HN, Cheang M, Kucab JE, Hsu FD, Rayaj R, et al. Expression of the insulin-like growth factor I receptor and urokinase plasminogen activator in breast cancer is associated with poor survival: potential for intervention with 17-allylamino geldanamycin. Cancer Res 2004;64:286-291.

32. Sachdev D, Hartell JS, Lee AV, Zhang X, Yee D. A dominant negative type I insulin-like growth factor receptor inhibits metastasis of human cancer cells. J Biol Chem 2004;279:5017-5024.

33. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004;4:505-518.

34. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell 2007;129:1261-1274.

35. Nicholoson KM, Anderson NG. The protein kinase B/Akt signalling pathway in human malignancy. Cell Signal 2002;14:381-395.

36. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Moynagh PN. The NF-kappaB pathway. Annu Rev Genomics Hum Genet 2004;5:505-518.

37. Bartlett JM, A’Hern R, Piper T, Ellis IO, Dowsett M, Mallon K, et al. Akt is frequently activated in HER2/neu-positive breast cancers and associated with poor prognosis among hormone-treated patients. Int J Cancer 2006;118:284-289.

38. Perez-Tenorio G, Stål O. Southeast Sweden Breast Cancer Group. Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. Br J Cancer 2002;86:540-545.

39. Vazquez F, Sellers WR. The PTEN tumor suppressor protein: an antagonist of phosphoinositide 3-kinase signaling. Biochim Biophys Acta 2000;1470:M21-M35.

40. Li J, Yen C, Liaw D, Podosypina K, Bose S, Wang SI, et al. Phosphorylation of AKT pathway proteins is not predictive of benefit of taxane therapy in early breast cancer. Breast Cancer Res Treat 2013;138:773-781.

41. Khan S, Kumagai T, Vora J, Bose N, Sebagal I, Koeffer PH, et al. PTEN promoter is methylated in a proportion of invasive breast cancers. Int J Cancer 2004;112:407-410.

42. Karkare S, Chhipa RR, Anderson J, Liu X, Henry H, Gasilina A, et al. Anti-proliferative and apoptosis inducing effects of nimbolide by altering molecules in PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943-1947.

43. Raja Singh P, Arunkumar R, Sivakamasundari V, Sharmila G, Elumalai P, Suganthapriya E, et al. Anti-proliferative and apoptosis inducing effect of nimbolide by altering molecules involved in apoptosis and IGF signalling via PI3K/Akt in prostate cancer (PC-3) cell line. Cell Biochem Funct 2014;32:217-228.

44. Moynagh PN. The NF-kappaB pathway. J Cell Sci 2005;118(Pt 20):4589-4592.

45. Kearns JD, Basak S, Werner SL, Huang CS, Hoffmann A.
IkappaBepsilon provides negative feedback to control NF-kappaB oscillations, signaling dynamics, and inflammatory gene expression. *J Cell Biol* 2006;173:659-664.

46. Elumalai P, Brindha Mercy A, Arunkumar R, Sharmila G, Bhat FA, Balakrishnan S, et al. Nimbolide inhibits invasion and migration, and down-regulates uPAR chemokine gene expression, in two breast cancer cell lines. *Cell Prolif* 2014;47:540-552.

47. Sachdev D. Regulation of breast cancer metastasis by IGF signaling. *J Mammary Gland Biol Neoplasia* 2008;13:431-441.

48. Bourboulia D, Stetler-Stevenson WG. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. *Semin Cancer Biol* 2010;20:161-168.

49. Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer* 2004;4:540-550.

50. Tang L, Han X. The urokinase plasminogen activator system in breast cancer invasion and metastasis. *Biomed Pharmacother* 2013;67:179-182.

51. Kong D, Li Y, Wang Z, Banerjee S, Sarkar FH. Inhibition of angiogenesis and invasion by 3,3’-diindolylmethane is mediated by the nuclear factor-kappaB downstream genes MMP-9 and uPA that regulated bioavailability of vascular endothelial growth factor in prostate cancer. *Cancer Res* 2007;67:3310-3319.

52. Sherr CJ. Cancer cell cycles. *Science* 1996;274:1672-1677.

53. Roy MK, Kobori M, Takenaka M, Nakahara K, Shinhoto M, Tsushida T. Inhibition of colon cancer (HT-29) cell proliferation by a triterpenoid isolated from *Azadirachta indica* is accompanied by cell cycle arrest and up-regulation of p21. *Planta Med* 2006;72:917-923.

54. Harish Kumar G, Chandra Mohan KV, Jagannadha Rao A, Nagini S. Nimbolide a limonoid from *Azadirachta indica* inhibits proliferation and induces apoptosis of human choriocarcinoma (BeWo) cells. *Invest New Drugs* 2009;27:246-252.

55. Priyadarsini NV, Murugan RS, Sripriya P, Karunagaran D, Nagini S. The neem limonoids azadirachtin and nimbolide induce cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells. *Free Radic Res* 2010;44:624-634.

56. Takahashi-Yanaga F, Sasaguri T. GSK-3beta regulates cyclin D1 expression: a new target for chemotherapy. *Cell Signal* 2008;20:581-589.

57. Yuan J, Yan R, Krämer A, Eckerdt F, Roller M, Kaufmann M, et al. Cyclin B1 depletion inhibits proliferation and induces apoptosis in human tumor cells. *Oncogene* 2004;23:5843-5852.

58. LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, et al. New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 1997;11:847-862.

59. Moldovan GL, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. *Cell* 2007;129:665-679.

60. Danial NN, Korsmeyer SJ. Cell death: critical control points. *Cell* 2004;116:205-219.

61. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, Griffith C, et al. Proliferation and apoptosis as markers of benefit in neoadjuvant endocrine therapy of breast cancer. *Clin Cancer Res* 2006;12(3 Pt 2):1024s-1030s.

62. Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. *Nat Rev Cancer* 2005;5:876-885.

63. Ashkenazi A, Herbst RS. To kill a tumor cell: the potential of proapoptotic receptor agonists. *J Clin Invest* 2008;118:1979-1990.

64. Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* 1999;6:99-104.

65. Zhou Y, Peng Y, Mao QQ, Li X, Chen MW, Su J, et al. Casticin induces caspase-mediated apoptosis via activation of mitochondrial pathway and upregulation of DR5 in human lung cancer cells. *Asian Pac J Trop Med* 2013;6:372-378.

66. Gupta SC, Reuter S, Phromnoi K, Park B, Hema PS, Nair M, et al. Nimbolide sensitizes human colon cancer cells to TRAIL through reactive oxygen species- and ERK-dependent up-regulation of death receptors, p53, and Bax. *J Biol Chem* 2011;286:1134-1146.