

## Recent study on the anticancer activity of nobiletin and its metabolites

Ke Lv<sup>a,b</sup>, Lingling Zhang<sup>a,b\*</sup>, Hui Zhao<sup>a\*</sup>, Chi-Tang Ho<sup>c</sup> and Shiming Li<sup>b</sup>

<sup>a</sup>Tianjin Key Laboratory of Food and Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China

<sup>b</sup>Hubei Key Laboratory of EFGIR, Huanggang Normal University, Huanggang, Hubei, 438000, China

<sup>c</sup>Department of Food Science, Rutgers University, New Brunswick, New Jersey 08901, United States

\*Corresponding author: Lingling Zhang and Hui Zhao, Tianjin Key Laboratory of Food and Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China; Hubei Key Laboratory of EFGIR, Huanggang Normal University, Huanggang, Hubei, 438000, China. E-mail: 1243536645@qq.com (LZ), zhaohui@tjcu.edu.cn (HZ)

DOI: 10.31665/JFB.2021.14267

Received: June 25, 2021; Revised received & accepted: June 30, 2021

Citation: Lv, K., Zhang, L., Zhao, H., Ho, C.-T., and Li, S. (2021). Recent study on the anticancer activity of nobiletin and its metabolites. J. Food Bioact. 14: 53–59.

### Abstract

The exploration of naturally occurring phytochemicals with antitumor potential has been the focus of many studies. Nobiletin, a polymethoxyflavone (PMF) exclusively derived from citrus peels, has been reported as a promising candidate for the prevention and/or treatment of cancers. Additionally, multiple demethylated derivatives of nobiletin from *in vivo* biotransformation, including 3'-demethylnobiletin (3'-DMN), 4'-demethylnobiletin (4'-DMN), 3',4'-didemethylnobiletin (3',4'-DDMN) and 5-demethylnobiletin (5-DMN), among others, have been found to show anti-cancer activity. In this review, the anticancer activity of nobiletin and its derivatives in cancer prevention are comprehensively described.

**Keywords:** Nobiletin; Cancer; Anti-Inflammation; Apoptosis; Autophagy.

### 1. Introduction

Cancer is becoming a major public concern because it is a leading cause of death worldwide. According to a recent statistic from the American Cancer Society, 1,898,160 new cancer cases and 608,570 cancer deaths are projected to occur in the United States in 2021 (Siegel et al., 2021). As a result of improvements in early detection and treatment, the cancer death rate dropped by 31% from its peak in 1991 until 2018 (Siegel et al., 2021). In recent years, attention has been focused on finding effective and low toxic anti-cancer agents as the alternative and complementary medicine for cancer treatment and prevention. Notably, accumulating data suggest that compounds from natural plants have been used to target cancer for decades because they usually have fewer side effects than non-natural anticancer drugs.

Nobiletin (5,6,7,8,3',4'-hexamethoxyflavone, Figure 1) is a natural flavonoid extracted exclusively from citrus peel (Wang

et al., 2018) and exhibits a good safety profile and a broad spectrum of pharmacological activities, including anti-inflammatory (Ozkan et al., 2020; Hagenlocher et al., 2019; Yang et al., 2019), antitumor (Ashrafizadeh et al., 2020) and neuroprotective activities (Amarsanaa et al., 2021). Not only does nobiletin itself exhibit anti-cancer properties, but also its *in vivo* metabolites, including 3'-demethylnobiletin (3'-DMN), 4'-demethylnobiletin (4'-DMN), 3',4'-didemethylnobiletin (3',4'-DDMN) and 5-demethylnobiletin (5-DMN) also have anti-cancer activity (Figure 1).

In this review, we summarize the anticancer activity of nobiletin and its derivatives in cancer therapy based on the newly published articles.

#### 1.1. Chemical aspects of nobiletin and its derivatives

Recently, nutritionists have shown interest in natural compounds extracted from plants and fruits in the prevention and treatment

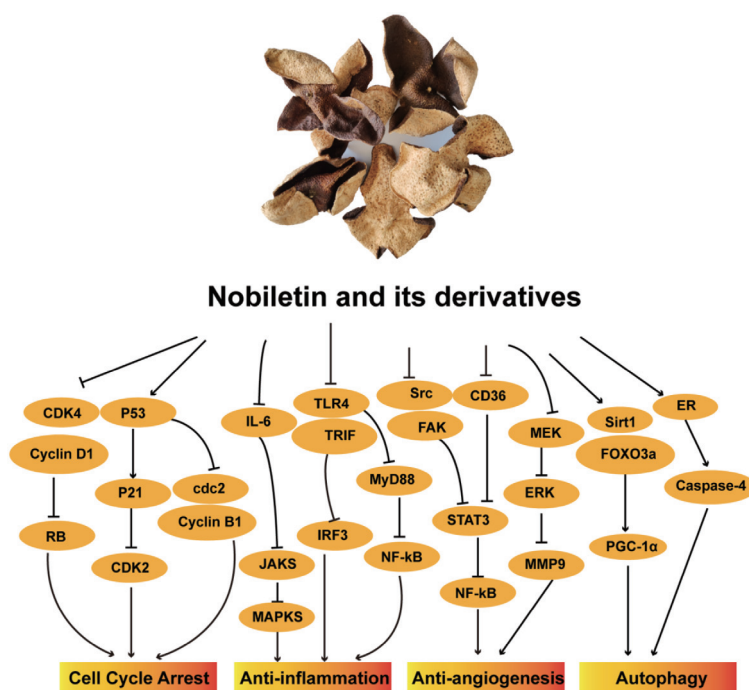


Figure 2. Schematic diagram showing the mechanisms underlying the anti-cancer effects of nobiletin and its derivatives through cell cycle arrest, anti-inflammation, anti-angiogenesis and induction of autophagy.

of various illnesses including cancer. Nobiletin can be extracted exclusively from citrus fruits, particularly in the peels of sweet oranges (*Citrus sinensis*) (Li, Yu and Ho, 2006) and mandarin oranges (*Citrus reticulata*) (Nogata et al., 2006) through different types of chemical extraction processes. There is concurrently ongoing research looking into the effect of nobiletin on different types of cancers, such as leukemia (Yen et al., 2020), colon cancer (Goh et al., 2019), breast cancer (Surichan et al., 2018), ovarian cancer (Zhang et al., 2020), thyroid cancer (Sousa et al., 2020), lung cancer (Da et al., 2016), and other tumor types. The metabolism of nobiletin comprises two phases, including phase I and phase II metabolism. Three common metabolites of nobiletin include 3'-DMN, 4'-DMN, and 3',4'-DDMN (Ashrafzadeh et al., 2020). Under long-term storage, nobiletin can be degraded to 5-DMN through the process of microbial autohydrolysis (Wang et al., 2018). Figure 1 illustrates

the chemical structures of nobiletin and its derivatives.

## 2. The anticancer activity of nobiletin and its derivatives

Dietary factors play key roles in the development and prevention of various diseases, including cancer. Nobiletin as a prospective anti-cancer agent for chemoprevention, is known to inhibit different pathways leading to cancer via a number of different mechanisms, which includes inhibiting cell cycle progression, limiting inflammation, inducing apoptosis, preventing angiogenesis and reducing tumor formation. Figure 2 schematizes the main anticarcinogenic pathways of nobiletin, its autohydrolysis product, 5-DMN and its three common metabolites, 3'-DMN, 4'-DMN, and 3',4'-DDMN, in chemoprevention of different cancers in detail and also are summarized in Table 1.

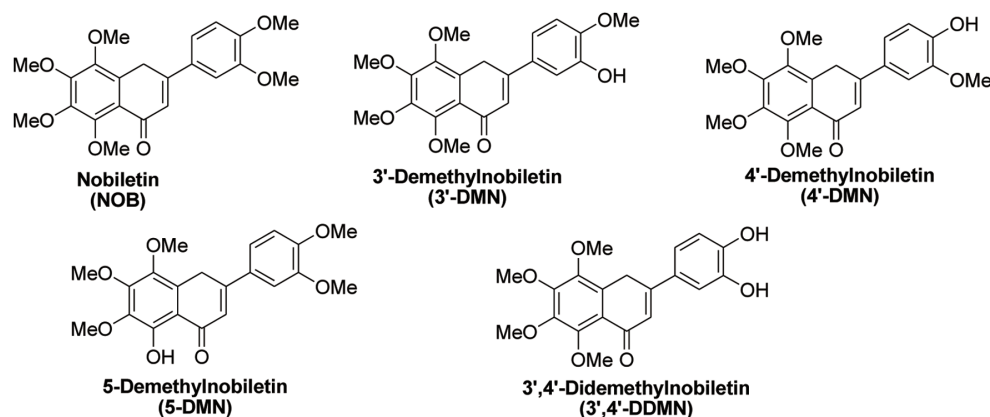


Figure 1. Structures of nobiletin and its *in vivo* metabolites.

Table 1. The anticancer activity of nobiletin and its derivatives

Protective Effects	PMF Compounds	Cancer Types	Dosage	Results	References
Cell Cycle Arrest	Nobiletin	Pancreatic cancer	6.12 µM	Inhibited the growth and metastasis. Induced autophagy. G0/G1 cell cycle arrest. Inhibited NF-κB signal pathway	Jiang et al., 2020
		Glioma	100 µM	Inhibited glioma cell growth and migration. Arrested the cell cycle in the G0/G1 phase. Suppressed MAPK and Akt pathways	Lien et al., 2016
	4'-DMN	Colon cancer	0.05% (w/w, in diet)	Inhibited cell growth. Induced G0/G1 cell cycle arrest and apoptosis	Wu et al., 2018
	3',4'-DDMN	Colon cancer	7.5 µM	Inhibited cell growth. Induced significant G2-M cell-cycle arrest and apoptosis	DiMarco-Crook et al., 2020
	5-DMN	Lung cancer	12.5 µM	Inhibited cell growth. Induced G2/M cell cycle phase arrest. Induced autophagy. Activated JNK signaling pathway	Chen et al., 2015
Anti-inflammation	4'-DMN, 3',4'-DDMN	Lung cancer	50 µM 25 µM	Inhibited cell growth caused cell cycle arrest and cellular apoptosis. Modulated the expression of proteins related to cell proliferation and death	Song et al., 2016
	Nobiletin	Prostate cancer	80 µM	Inhibited cell growth. Suppressed TLR4/TRIF/IRF3, TLR9/IRF7 signal pathways. Inhibited the release of IFN-α and IFN-β	Ozkan et al., 2020
	Nobiletin and its metabolites	Colon cancer	2.03 µM, NOB; 3.28 µM, 3'-DMN; 24.13 µM, 4'-DMN; 12.03 µM, 3',4'-DDMN	Suppressed LPS-induced iNOS and COX-2 expression. Increased heme oxygenase-1 (HO-1) and NQO1 expression	Wu et al., 2017
Anti-angiogenesis	3',4'-DDMN	Skin tumor	1 or 3 µM	Inhibited TPA-induced iNOS, COX-2, VEGF, MMP-9 and ODC expression, nuclear translocation and phosphorylation of p65/RelA	Lai et al., 2008
	Nobiletin	Breast cancer	200 µM	Inhibited cell growth. Inhibited VEGF-dependent angiogenesis. Inhibited EGFR activity and Src/FAK/STAT3 signaling	Sp et al., 2017
				Inhibited the expression of CD36 and its downstream target proteins. Inhibited CD36-mediated Angiogenesis. Inhibited CD36-dependent breast cancer cell migration and invasion, inhibited tumorsphere formation and survival	Sp et al., 2018
	Nobiletin	Colorectal cancer	64 µM	Inhibited MEK activity and ERK phosphorylation	Miyata et al., 2008
Autophagy	Nobiletin	Hepatic Ischemia and reperfusion injury	5 mg/kg	Alleviated oxidative damage, inflammation, and cell death caused by hepatic ischemia and reperfusion. Activated the SIRT-1/FOXO3a and PGC-1α pathways	Dusabimana et al., 2019
		Ovarian cancer	40 µM	Selectively suppressed cell growth and proliferation. Induced G0/G1 phase arrest and reduced G2/M phase. Induced apoptosis.	Jiang et al., 2018

(continued)

Table 1. (continued)

Protective Effects	PMF Compounds	Cancer Types	Dosage	Results	References
	Nobiletin	Gastric cancer	50 $\mu$ M	Increased the levels of ER-stress related proteins IRE1- $\alpha$ , ATF-4, C/EBP homology protein, GRP78 and caspase-4. Decreased cell viability. Increased the number of sub-G1 phase cells. Induced apoptosis	Moons and Cho, 2016
	5-DMN	Colon cancer	50 or 100 mg/kg	Decreased the tumor size and tumor weight. Increased the ratio of cleaved-PARP and LC3 expression in tumor tissues. Induced p53-regulated cell death signaling pathway	Song et al., 2020
ROS	Nobiletin	Ovarian Cancer	50 $\mu$ M	Inhibited cell growth. Induced cell apoptosis and autophagy. Induced ROS generation contributing to pyroptosis	Zhang et al., 2020
		Oral cancer	100 $\mu$ M	Increased the expression of the apoptosis molecule PARP and caspase-3. Increased the level of ROS and the degree of DNA damage. Inhibited cell growth	Yang et al., 2020
		Gastric cancer	50 $\mu$ M	Reduced ROS levels. Inhibited p38, extracellular receptor kinase 1, and c-Jun amino-terminal expression. Inhibited cell growth	Ouyang, Li and Ling, 2020

### 2.1. Cell cycle arrest

The cell cycle is a complex sequence of events through which a cell duplicates its contents then divides to two genetically identical daughter cells. Mutations and deficiencies in regulation throughout the cell cycle can lead to serious diseases such as cancer (Wenzel and Singh, 2018). Therefore, one way to suppress cancer is to block its cell cycle progression. Studies in recent years elucidated that nobiletin inhibited cancer behavior through cell cycle arrest. For example, Jiang et al. (2020) demonstrated that nobiletin inhibits the growth and metastasis of human pancreatic cancer cells via induction of G0/G1 cell cycle arrest and showed an IC50 of 6.12  $\mu$ M. In glioma cell, the flow cytometry test showed that 50 and 100  $\mu$ M nobiletin induced dose-dependently increase in the G0/G1 subpopulation by approximately 8.7 and 17.2%, respectively, compared with the solvent control (0.5% DMSO), indicating that nobiletin can arrest the cell cycle in the G0/G1 phase (Lien et al., 2016). Combining different chemopreventive agents is a promising strategy to reduce cancer incidence and mortality due to potential synergistic interactions between these agents. Co-treatment with 4'-DMN and atorvastatin (a lipoprotein-lowering drug) strongly inhibited the growth of human colon cancer cells by inducing G0/G1 cell cycle arrest and apoptosis (Wu et al., 2018). In HCT116 human colon cancer cells, the combination of curcumin and 3',4'-DDMN, a primary metabolite of nobiletin, induced significant G2/M cell-cycle arrest and extensive apoptosis, which greatly exceeded the effects of individual treatments with curcumin or 3',4'-DDMN (DiMarco-Crook et al., 2020). *In vitro* tests using lung cancer cells revealed 5-DMN, 4'-DMN, and 3',4'-DDMN inhibited cell proliferation by inducing G2/M cell cycle phase arrest (Chen et al., 2015, Song et al., 2016).

### 2.2. Anti-Inflammation

Inflammation is the body's response to tissue damage, caused by physical injury, ischemic injury, infection, exposure to toxins, or other types of trauma. Inflammation is often associated with the development and progression of cancer. The triggers of chronic inflammation that increase cancer risk or progression include infections, autoimmune diseases, and inflammatory conditions of uncertain origin (Singh et al., 2019). In addition, cancer cells as well as surrounding stromal and inflammatory cells engage in well-orchestrated reciprocal interactions to form an inflammatory tumor microenvironment (Gretten and Grivennikov, 2019).

Increasing evidence showed that inflammatory cytokines and chemokines, which can be produced by the tumor cells and/or tumor-associated leukocytes and platelets, may contribute directly to malignant progression. Therefore, one way to suppress cancer is to inhibit the secretion of pro-inflammatory cytokines. Ozkan et al. (2020) reported that the anti-inflammatory effect of nobiletin on TLR4/TRIF/IRF3 and TLR9/IRF7 signaling pathways in PCa cells for the first time. Treatment with nobiletin resulted in a noteworthy reduction of cell growth and the release of IFN- $\alpha$  and IFN- $\beta$ . Besides nobiletin, multiple studies have shown that its metabolites, especially, 4'-DMN, and 3',4'-DDMN also exhibit significant anti-inflammation effect towards nitric oxide production, iNOS and cyclooxygenase (COX) expressions in cancer (Wu et al., 2017). By administering nobiletin and its metabolites at a concentration equivalent in the LPS-induced RAW 246.7 macrophages decreased iNOS and COX-2 protein expression levels and induced heme oxygenase-1 (HO-1) protein expression (Wu et al., 2017). Additionally, the anti-inflammatory activity of 3',4'-DDMN can be

demonstrated by its effect on iNOS, COX-2, VEGF, matrix metalloproteinase-9 (MMP-9) and ODC expression in TPA-stimulated mouse skin (Lai et al., 2008). iNOS and COX-2 are frequently regulated by activating the NF- $\kappa$ B signaling pathway (Chun et al., 2004). 3',4'-DDMN treatment caused a significant reduction of TPA-induced nuclear translocation and phosphorylation of p65/RelA, the functionally active subunit of NF- $\kappa$ B protein (Lai et al., 2008).

### 2.3. Anti-angiogenesis

Angiogenesis is a biological process in which novel blood vessels are formed, providing tissues with oxygen and nutrients. Folkman (1971) first proposed that angiogenesis was related to tumor growth, forming the hypothesis of targeting angiogenesis to treat cancer. Since then, many efforts have been made to explore the possibility of curing cancer by targeting angiogenesis.

Steroid receptor coactivator (Src) and focal adhesion kinase (FAK) are intracellular tyrosine kinases that play an important role in tumor angiogenesis. Nobiletin inhibited tumor angiogenesis by regulating Src, FAK, and STAT3 signaling through PXN in ER<sup>+</sup> breast cancer cells (Sp et al., 2017). CD36 is implicated in tumor metastasis through its roles in fatty acid metabolism. Recent research showed that nobiletin inhibited angiogenesis by suppressing CD36 expression and decreasing the expression of TSP-1 and TGF- $\beta$ 1 (Sp et al., 2018). In the zebrafish angiogenesis model, nobiletin showed anti-angiogenic activity by blocking new blood vessel formation (Lam et al., 2011). Leptin is thought to be a mitogenic factor that leads to the development of colon cancer. Treatment with nobiletin suppressed cell proliferation induced by leptin through inhibition of mitogen-activated protein/extracellular signal-regulated kinase (MEK) 1/2 (Miyata et al., 2008).

### 2.4. Autophagy

Autophagy plays a dichotomous role in cancer by suppressing benign tumor growth but promoting advanced cancer growth. In the past decade, numerous researches have established autophagy as a potential therapeutic target in cancer.

Hepatic ischemia and reperfusion injury are characterized by impaired autophagy, mitochondrial dysfunction, and subsequent damage of cellular homeostasis after hepatic surgery or transplantation. Treatment with nobiletin (5 mg/kg) ameliorated oxidative damage, inflammation, and cell death caused by hepatic ischemia and reperfusion through the activation of SIRT1/FOXO3a-mediated autophagy and mitochondrial biogenesis (Dusabimana et al., 2019). Autophagy is a cellular defense mechanism involving the degradation and recycling of cytoplasmic constituents. Nobiletin suppressed autophagic degradation via over-expressing AKT pathway and enhances apoptosis in multidrug-resistant SKOV3/TAX ovarian cancer cells (Jiang et al., 2018). ER stress-induced apoptotic signaling has been studied to identify potential targets for therapeutic intervention in diseases associated with ER stress (Puthalakath et al., 2007). Studies have shown that nobiletin-induced apoptosis in SNU-16 cells was mediated by pathways involving intracellular ER stress-mediated protective autophagy (Moon and Cho, 2016). Notably, different metabolites of NOB work by different mechanisms against different cancer. In vivo tests using xenograft mouse model showed that 5-DMN exhibited more preferential anti-colon cancer effects than NOB via its ability to induce p53-regulated autophagy

(Song et al., 2020).

### 2.5. Reactive oxygen species (ROS)

Extensive research indicated that reactive oxygen species (ROS), including superoxide radical, hydroxyl radical, and hydrogen peroxide, play an important role in cancer. Although low levels of ROS can be beneficial, excessive accumulation can promote cancer. For example, they can activate pro-tumorigenic signaling, enhance cell survival and proliferation, and drive DNA damage and genetic instability (Diao et al., 2016; Prasad et al., 2017; Sarsour et al., 2008). However, excess ROS can also promote anti-tumorigenic signaling, initiating oxidative stress-induced tumor cell death (Liou and Storz, 2010; Roy et al., 2015; Stanicka et al., 2015). Anti-tumorigenic signaling of ROS can be targeted as a therapy in cancer, by the increased production of ROS levels to toxic levels and exhaustion of the antioxidant system capacity causing programmed cell death. Numerous studies from preclinical to clinical models have indicated that compounds from natural plants are rich in antioxidants provided a protective effect in the development of cancer (Li et al., 2020; Wang et al., 2018; Zhang et al., 2018). Pyroptosis is a pro-inflammatory form of programmed cell death that is triggered by a series of cytosolic sensors to induce cancer cell death (Man, Karki and Kanneganti, 2017). Since nobiletin can trigger ROS-mediated pyroptosis, it is considered a promising new anti-ovarian cancer drug candidate (Zhang et al., 2020). In addition, the intracellular ROS level was dose-dependent with the increase of NOB concentration (Yang et al., 2020).

Inhibition of ROS production in tumor cells could well result in suppressed pro-tumorigenic signaling. ROS are also thought to be involved in inflammatory gene expression through the activation of NF- $\kappa$ B signaling pathway (Kabe et al., 2005). Studies have shown that nobiletin inhibited the DNA-binding activity of NF- $\kappa$ B and ROS production in RAW 264.7 cells (Choi et al., 2007). Treatment with nobiletin reduced ROS levels and apoptotic morphological changes and decreased levels of lipid peroxides and glutathione content in H. pylori-infected GES-1 cells (Ouyang, Li and Ling, 2020).

It is becoming increasingly evident that ROS play an important role in carcinogenesis. Such dual roles of ROS indicate their critical importance in cellular homeostasis. Therefore, targeting ROS could be a promising approach for cancer therapeutics.

## 3. Conclusions

Overall, there is mounting interest in the therapeutic utility of nobiletin and its autohydrolysis product 5-DMN and several metabolites of nobiletin such as 3'-DMN, 4'-DMN, and 3',4'-DDMN. However, biopharmaceutical limitations including low aqueous solubility, poor permeability across biological barriers and low bioavailability hinder further accessibility for targeting hard-to-reach cancers. Efforts in exploring and developing effective strategies to overcome the biopharmaceutical challenges associated with nobiletin seems to be a promising way to enhance its therapeutic effects.

## Acknowledgments

This work was supported by the Grant from Hubei Province, China (GRANT number 2019ABA100).

## References

- Amarsanaa, K., Kim, H.J., KO, E.-A., Jo, J., and Jung, S.-C. (2021). Nobiletin exhibits neuroprotective effects against mitochondrial complex I inhibition via regulating apoptotic signaling. *Exp. Neurobiol.* 30(1): 73–86.
- Ashrafizadeh, M., Zarrabi, A., Saberifar, S., Hashimi, F., Hushmandi, K., Hashemi, F., Mogadam, E.R., Mohammadinejad, R., Najafi, M., and Garg, M. (2020). Nobiletin in cancer therapy: how this plant derived-natural compound targets various oncogene and onco-suppressor pathways. *Biomedicines* 8(5): 110.
- Chen, Y.-K., Wang, H.-C., Ho, C.-T., Chen, H.-Y., Li, S., Chan, H.-L., Chung, T.-W., Tan, K.-T., Li, Y.-R., and Lin, C.-C. (2015). 5-Demethylnobiletin promotes the formation of polymerized tubulin, leads to G2/M phase arrest and induces autophagy via JNK activation in human lung cancer cells. *J. Nutr. Biochem.* 26(5): 484–504.
- Choi, Y., Hwang, J.-H., Ko, H.-C., Park, J.-G., and Kim, S.-J. (2007). Nobiletin from citrus fruit peel inhibits the DNA-binding activity of NF-kappa B and ROS production in LPS-activated RAW 264.7 cells. *J. Ethnopharmacol.* 113(1): 149–155.
- Chun, K.-S., Cha, H.-H., Shin, J.-W., Na, H.-K., Park, K.-K., Chung, W.-Y., and Surh, Y.-J. (2004). Nitric oxide induces expression of cyclooxygenase-2 in mouse skin through activation of NF-kappaB. *Carcinogenesis* 25(3): 445–454.
- Da, C., Liu, Y., Zhan, I., Liu, K., and Wang, R. (2016). Nobiletin inhibits epithelial-mesenchymal transition of human non-small cell lung cancer cells by antagonizing the TGF-beta1/Smad3 signaling pathway. *Oncol. Rep.* 35(5): 2767–2774.
- Diao, Q.X., Zhang, J.Z., Zhao, T., Xue, Z.F., Ma, S.M., and Wang, Y. (2016). Vitamin E promotes breast cancer cell proliferation by reducing ROS production and p53 expression. *Eur. Rev. Med. Pharmacol.* 20(12): 2710–2717.
- DiMarco-Crook, C., Rakariyatham, K., Li, Z., Du, Z., Zheng, J., Wu, X., and Xiao, H. (2020). Synergistic anticancer effects of curcumin and 3',4'-didemethylnobiletin in combination on colon cancer cells. *J. Food Sci.* 85(4): 1292–1301.
- Dusabimana, T., Kim, S.R., Kim, S.J., Park, S.W., and Kim, H. (2019). Nobiletin ameliorates hepatic ischemia and reperfusion injury through the activation of SIRT-1/FOXO3a-mediated autophagy and mitochondrial biogenesis. *Exp. Mol. Med.* 51: 1–16.
- Folkman, J. (1971). Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* 285(21): 1182–1186.
- Goh, J.X.H., Tan, L.T.H., Goh, J.K., Chan, K.G., Pasparajah, P., Lee, L.-H., and Goh, B.-H. (2019). Nobiletin and derivatives: functional compounds from citrus fruit Peel for colon cancer chemoprevention. *Cancers.* 11(6): 867.
- Greten, F.R., and Grivennikov, S.I. (2019). Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity.* 51(1): 27–41.
- Hagenlocher, Y., Gommeringer, S., Held, A., Feihauer, K., Konginger, J., Bischoff, S.C., and Lorentz, A. (2019). Nobiletin acts anti-inflammatory on murine IL-10(-/-) colitis and human intestinal fibroblasts. *Eur. J. Nutr.* 58(4): 1391–401.
- Jiang, H., Chen, H., Jin, C., Mo, J., and Wang, H. (2020). Nobiletin flavone inhibits the growth and metastasis of human pancreatic cancer cells via induction of autophagy, G0/G1 cell cycle arrest and inhibition of NF-kB signalling pathway. *J. Buon.* 25(2): 1070–1075.
- Jiang, Y.P., Guo, H., and Wang, X.B. (2018). Nobiletin (NOB) suppresses autophagic degradation via over-expressing AKT pathway and enhances apoptosis in multidrug-resistant SKOV3/TAX ovarian cancer cells. *Biomed. Pharmacother.* 103: 29–37.
- Kabe, Y., Ando, K., Hirao, S., Yoshida, M., and Handa, H. (2005). Redox regulation of NF-kappa B activation: Distinct redox regulation between the cytoplasm and the nucleus. *Antioxid. Redox. Sign.* 7(3-4): 395–403.
- Lai, C.S., Li, S., Chai, C.Y., Lo, C.-Y., Sushenkov, S., Ho, C.-T., Pan, M.-H., and Wang, Y.-J. (2008). Anti-inflammatory and antitumor promotional effects of a novel urinary metabolite, 3',4'-didemethylnobiletin, derived from nobiletin. *Carcinogenesis* 29(12): 2415–2424.
- Lam, K.H., Alex, D., Lam, I.K., Tsui, W.S.K., Yang, Z.F., and Lee, S.M.Y. (2011). Nobiletin, a polymethoxylated flavonoid from citrus, shows anti-angiogenic activity in a zebrafish in vivo model and HUVEC in vitro model. *J. Cell Biochem.* 112(11): 3313–3321.
- Li, M., Zhao, H., Wu, J., Wang, L., Wang, J., Lv, K., Liu, S., Wang, M., Guan, W., Liu, J., Ho, C.-T., and Li, S. (2020). Nobiletin protects against acute liver injury via targeting c-Jun N-terminal kinase (JNK)-induced apoptosis of hepatocytes. *J. Agric. Food Chem.* 68(27): 7112–7120.
- Li, S., Yu, H., and Ho, C.-T. (2006). Nobiletin: efficient and large quantity isolation from orange peel extract. *Biomed. Chromatogr.* 20(1): 133–138.
- Lien, L.M., Wang, M.J., Chen, R.J., Chiu, H.-C., Wu, J.-L., Shen, M.-Y., Chou, D.-S., Sheu, J.-R., Lin, K.-H., and Lu, W.-J. (2016). Nobiletin, a polymethoxylated flavone, inhibits glioma cell growth and migration via arresting cell cycle and suppressing MAPK and Akt pathways. *Phytother. Res.* 30(2): 214–221.
- Liou, G.Y., and Storz, P. (2010). Reactive oxygen species in cancer. *Free Radic. Res.* 44(5): 479–496.
- Man, S.M., Karki, R., and Kanneganti, T.D. (2017). Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol. Rev.* 277(1): 61–75.
- Miyata, Y., Sato, T., Imada, K., Dobashi, A., Yano, M., and Ito, A. (2008). A citrus polymethoxyflavonoid, nobiletin, is a novel MEK inhibitor that exhibits antitumor metastasis in human fibrosarcoma HT-1080 cells. *Biochem. Biophys. Res. Commun.* 366(1): 168–173.
- Moon, J.Y., and Cho, S.K. (2016). Nobiletin induces protective autophagy accompanied by ER-stress mediated apoptosis in human gastric cancer SNU-16 cells. *Molecules* 21(7): 914.
- Nogata, Y., Sakamoto, K., Shiratsuchi, H., Ishii, T., Yano, M., and Ohta, H. (2006). Flavonoid composition of fruit tissues of Citrus species. *Biosci. Biotech. Bioch.* 70(1): 178–192.
- Ouyang, Y., Li, L., and Ling, P. (2020). Nobiletin inhibits helicobacterium pylori infection-induced gastric carcinogenic signaling by blocking inflammation, apoptosis, and mitogen-activated protein kinase events in gastric epithelial-1 cells. *J. Environ. Pathol. Tox.* 39(1): 77–88.
- Ozkan, A.D., Kaleli, S., Onen, H.I., Sarihan, M., Eskiler, G.G., Yigin, A.K., and Akdogan, M. (2020). Anti-inflammatory effects of nobiletin on TLR4/TRIF/IRF3 and TLR9/IRF7 signaling pathways in prostate cancer cells. *Immunopharmacol. Immunotoxicol.* 42(2): 93–100.
- Prasad, S., Gupta, S.C., and Tyagi, A.K. (2017). Reactive oxygen species (ROS) and cancer: role of antioxidative nutraceuticals. *Cancer Lett.* 387: 95–105.
- Puthalakath, H., O'Reilly, L.A., Gunn, P., Lee, L., Kelly, P.N., Huntington, N.D., Hughes, P.D., Michalak, E.M., McKimm-Brechkin, J., Motoyama, N., Gotoh, T., Akira, S., Bouillet, P., and Strasser, A. (2007). ER stress triggers apoptosis by activating BH3-only protein Bim. *Cell* 129(7): 1337–1349.
- Roy, K., Wu, Y., Meitzler, J.L., Juhasz, A., Liu, H., Jiang, G., Lu, J., Antony, S., and Soroshov, J.H. (2015). NADPH oxidases and cancer. *Clin. Sci.* 128(12): 863–875.
- Sarsour, E.H., Venkataraman, S., Kalen, A.L., Oberley, L.W., and Goswami, P.C. (2008). Manganese superoxide dismutase activity regulates transitions between quiescent and proliferative growth. *Aging Cell.* 7(3): 405–417.
- Siegel, R.L., Miller, K.D., Fuchs, H.E., and Jemal, A.D.V.M. (2021). Cancer Statistics, 2021. *CA Cancer J. Clin.* 71(1): 7–33.
- Singh, N., Baby, D., Rajguru, J.P., Patil, P.B., Thakkannavar, S.S., and Pujari, V.P. (2019). Inflammation and cancer. *Ann. Afr. Med.* 18(3): 121–126.
- Song, M., Charoensinphon, N., Wu, X., Zheng, J., Gao, Z., Xu, F., Wang, M., and Xiao, H. (2016). Inhibitory effects of metabolites of 5-demethylnobiletin on human nonsmall cell lung cancer cells. *J. Agr. Food Chem.* 64(24): 4943–4949.
- Song, M., Lan, Y., Wu, X., Han, Y., Wang, M., Zheng, J., Li, Z., Li, F., Zhou, J., Cao, Y., and Xiao, H. (2020). The chemopreventive effect of 5-demethylnobiletin, a unique citrus flavonoid, on colitis-driven colorectal carcinogenesis in mice is associated with its colonic metabolites. *Food Funct.* 11(6): 4940–4952.
- Sousa, D.P., Pojo, M., Pinto, A.T., Leite, V., Serra, A.T., and Cavaco, B.M. (2020). Nobiletin alone or in combination with cisplatin decreases the viability of anaplastic thyroid cancer cell lines. *Nutr. Cancer.* 72(2): 352–363.
- Sp, N., Kang, D.Y., Joung, Y.H., Park, J.H., Kim, W.S., Lee, H.K., Song, K.-D., Park, Y.-M., and Yang, Y.M. (2017). Nobiletin inhibits angiogenesis

- by regulating Src/FAK/STAT3-mediated signaling through PXN in ER+ breast cancer cells. *Int. J. Mol. Sci.* 18(5): 935.
- Sp, N., Kang, D.Y., Joung, Y.H., Kim, D.H., Park, J.H., Lee, H.G., Kim, H.J., Darvin, P., Park, Y.-M., and Yang, Y.M. (2018). Nobiletin inhibits CD36-dependent tumor angiogenesis, migration, invasion, and sphere formation through the Cd36/Stat3/Nf-Kb signaling axis. *Nutrients* 10(6): 772.
- Stanicka, J., Russell, E.G., Woolley, J.F., and Cotter, T.G. (2015). NADPH Oxidase-generated hydrogen peroxide induces DNA damage in mutant FLT3-expressing leukemia cells. *J. Biol. Chem.* 290(15): 9348–9361.
- Surichan, S., Arroo, R.R., Ruparelia, K., Tsatsakis, A.M., and Androutsopoulos, V.P. (2018). Nobiletin bioactivation in MDA-MB-468 breast cancer cells by cytochrome P450 CYP1 enzymes. *Food Chem. Toxicol.* 113: 228–235.
- Wang, M., Meng, D., Zhang, P., Wang, X., Du, G., Brennan, C., Li, S., Ho, C.-T., and Zhao, H. (2018). Antioxidant protection of nobiletin, 5-demethylnobiletin, tangeretin, and 5-demethyltangeretin from citrus peel in *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* 66(12): 3155–60.
- Wenzel, E.S., and Singh, A.T.K. (2018). Cell-cycle checkpoints and aneuploidy on the path to cancer. *In Vivo.* 32(1): 1–5.
- Wu, X., Song, M., Gao, Z., Sun, Y., Wang, M., Li, F., Zheng, J., and Xiao, H. (2017). Nobiletin and its colonic metabolites suppress colitis-associated colon carcinogenesis by down-regulating iNOS, inducing antioxidative enzymes and arresting cell cycle progression. *J. Nutr. Biochem.* 42: 17–25.
- Wu, X., Song, M., Qiu, P., Li, F., Wang, M., Zheng, J., Wang, Q., Xu, F., and Xiao, H. (2018). A metabolite of nobiletin, 4'-demethylnobiletin and atorvastatin synergistically inhibits human colon cancer cell growth by inducing G0/G1 cell cycle arrest and apoptosis. *Food Funct.* 9(1): 87–95.
- Yang, G., Lin, C.C., Yang, Y., Yuan, L., Wang, P., Wen, X., Pan, M.H., Zhao, H., Ho, C.-T., and Li, S. (2019). Nobiletin prevents trimethylamine oxide-induced vascular inflammation via inhibition of the NF-kappaB/ MAPK pathways. *J. Agric. Food Chem.* 67(22): 6169–76.
- Yang, J., Yang, Y., Wang, L., Jin, Q., and Pan, M. (2020). Nobiletin selectively inhibits oral cancer cell growth by promoting apoptosis and DNA damage in vitro. *Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol.* 130(4): 419–427.
- Yen, J.-H., Lin, C.-Y., Chuang, C.-H., Chin, H.-K., Wu, M.-J., and Chen, P.-Y. (2020). Nobiletin promotes megakaryocytic differentiation through the MAPK/ERK-dependent EGR1 expression and exerts anti-leukemic effects in human chronic myeloid leukemia (CML) K562 cells. *Cells* 9(4): 877.
- Zhang, L., Wen, X., Li, M., Li, S., and Zhao, H. (2018). Targeting cancer stem cells and signaling pathways by resveratrol and pterostilbene. *Biofactors* 44(1): 61–68.
- Zhang, R., Chen, J., Mao, L., Guo, Y., Hao, Y., Deng, Y., Han, X., Li, Q., Liao, W., and Yuan, M. (2020). Nobiletin triggers reactive oxygen species-mediated pyroptosis through regulating autophagy in ovarian cancer cells. *J. Agr. Food Chem.* 68(5): 1326–1336.
- Zhou, C.B., and Fang, J.Y. (2019). The role of pyroptosis in gastrointestinal cancer and immune responses to intestinal microbial infection. *Bba-Rev. Cancer.* 1872(1): 1–10.