

Xanthohumol from Hops Prevents Hormone-Dependent Tumourigenesis In Vitro and In Vivo

J. Strathmann, E. Bertl, R. Hussong, K. Klimo, R. Steinle, N. Frank and C. Gerhauser
Division of Epigenomics and Cancer Risk Factors
Workgroup Chemoprevention
German Cancer Research Center
Heidelberg
Germany

Keywords: *Humulus lupulus* L., cancer chemoprevention, anti-estrogenic, anti-angiogenic, DMBA-induced mammary carcinogenesis, rat uterotrophic assay

Abstract

Since the 1990s, interest in health-promoting effects of xanthohumol (XN) has increased constantly. This manuscript will review some of the published literature as well as novel findings on XN. In 2002, we identified XN as a potent cancer chemopreventive agent acting by multiple mechanisms relevant for the prevention of carcinogenesis. Although hops is commonly linked with phytoestrogenic effects, we identified XN as a pure estrogen antagonist. Interestingly, XN may also reduce the generation of estrogens by inhibition of the enzymatic activity of aromatase, which converts testosterone to estrogen. Anti-estrogenic effects of XN (100 mg/kg body weight/day) were confirmed in vivo in an uterotrophy assay with prepubertal rats. In two fertility studies, long-term treatment with XN did not cause any adverse effect on female reproduction and on the development of offspring when given either for four weeks prior to or during mating, gestation and nursing. Novel data indicate that XN (100 mg/kg body weight/day in drinking water) prevents dimethylbenz[*a*]anthracene (DMBA)-induced mammary carcinogenesis in rats when given nine days prior to and for 16 weeks after the carcinogen. Tumour incidence was not influenced by XN treatment, but XN significantly reduced tumour multiplicity and tumour burden. XN (1000 mg/kg body weight/day injected subcutaneously for 14 days) also significantly reduced the growth of human breast cancer xenotransplants in immune-compromised mice and reduced tumour-induced neovascularization. Overall, these data demonstrate breast cancer preventive efficacy of XN. Multiple mechanisms, including modulation of DMBA metabolism, anti-estrogenic, anti-proliferative and anti-angiogenic activities may account for these effects and are under further investigation.

INTRODUCTION

Chemoprevention is the process of inhibiting, delaying or reversing carcinogenesis in the premalignant phase; it aims to halt or reverse the development and progression of precancerous cells to invasive cancer through use of non-toxic nutrients, phytochemicals and synthetic pharmacological agents (Sporn and Newton, 1979). These can act during the time from initiation to progression of tumours. The development of cancer is a multi-stage process which is broadly divided into initiation, promotion and progression phases (Fig. 1). Carcinogenesis may be regarded as a continuous accumulation of genetic and biochemical cell damage and offers multiple molecular targets for chemopreventive agents to prevent, inhibit or slow the progression from early genetic lesions to invasive tumour development (De Flora and Ferguson, 2005). In the initiation phase, reactive oxygen species (ROS), mutagens, or carcinogens, either directly or after metabolic activation to reactive molecules, interact with intracellular macromolecules. This may cause DNA damage, which can result in genetic and other cellular damage if not repaired. Repeated contact to tumour-promoters during the promotion phase may result in modified cell structure, uncontrolled cell proliferation, tumour growth and metastases. Consequently, for the identification of novel chemopreventive agents, we have set up a

series of cell- and enzyme-based in vitro assay systems to measure chemopreventive potential of isolated compounds and during activity-guided fractionation of crude extracts. Mechanisms include the modulation of drug metabolism, anti-oxidant, radical-scavenging, anti-inflammatory, anti-tumour promoting and anti-proliferative activities as well as induction of terminal cell differentiation and apoptosis (Gerhauser et al., 2003).

Why test hops for cancer chemopreventive potential? Hops (*Humulus lupulus* L.) are an important source of phenolic compounds in beer. The spectrum of polyphenols includes phenolcarbonic acids, prenylated flavonoids and chalcones, catechins and proanthocyanidins. In the 1990s, prenylated flavonoids from hops were shown to potentially influence carcinogenesis by modulation of carcinogen metabolism in vitro, as well as by anti-oxidant, anti-proliferative and cytotoxic effects. In addition, 8-prenylnaringenin was identified as a potent phytoestrogen (reviewed in Gerhauser, 2005a). These reports prompted us to fractionate a polyvinylpyrrolidone residue recovered as a waste product during brewing, an unfiltered lager type beer (Gerhauser et al., 2002b), and finally hops extracts (Gerhauser et al., 2002a) to analyze them for chemopreventive potential.

Here, we will summarize some of our published data as well results from other groups, and briefly report some of our recent results obtained with xanthohumol (XN, Fig. 1).

BROAD-SPECTRUM CANCER-CHEMOPREVENTIVE POTENTIAL OF XN IN VITRO

As a major outcome of our activity-guided fractionation of 300 L of lager-type beer, we isolated the prenylated chalcone XN as a novel broad-spectrum cancer chemopreventive lead structure (Gerhauser et al., 2002b) with activities at all stages of carcinogenesis (Gerhauser et al., 2002a) (Fig. 1, right side). Of note, XN and related prenylated chalcones are now available via a synthetic approach (Khupse and Erhardt, 2007; Vogel and Heilmann, 2008; Vogel et al., 2008). Anti-initiating mechanisms include antigenotoxic and antimutagenic activity (Kac et al., 2003; Plazar et al., 2007, 2008). Also, XN modulates activity of enzymes involved in carcinogen metabolism and detoxification (Gerhauser et al., 2002a). In addition, XN is able to scavenge a variety of physiological relevant radicals including peroxy-, hydroxyl-, and superoxide anion-radicals (Gerhauser et al., 2002a; also in Vogel et al., 2008). XN was characterized as an anti-inflammatory agent. It was found to inhibit both cyclooxygenases Cox-1 and Cox-2 activities and was shown to decrease LPS-mediated inducible nitric oxide synthase (iNOS) induction in cultured Raw 264.7 murine macrophages (Gerhauser et al., 2002a). This was confirmed recently (Cho et al., 2008). Using alkaline phosphatase induction in the Ishikawa cell line, XN was identified as an anti-estrogen without possessing estrogenic potential (see below) (Gerhauser et al., 2002a). It also inhibits aromatase (Cyp19) activity (Fig. 4, Strathmann et al., in preparation) (Monteiro et al., 2006, 2007). With respect to anti-proliferative mechanisms, XN was shown to decrease human recombinant DNA polymerase α activity, and to inhibit DNA synthesis in MDA MB 435 human breast cancer cells (Gerhauser et al., 2002a). Cytotoxic activity of XN has recently been associated with an inhibition of topoisomerase I activity (Lee et al., 2007). Poly(ADP-ribose)polymerase (PARP) cleavage, activation of caspases-3, -7, -8, and 9 and downregulation of Bcl-2 protein expression were found to contribute to apoptosis induction in cultured human colon cancer cells (Pan et al., 2005). XN also induced apoptosis in several other cancer cell lines, including leukemia cell lines (Dell'Eva et al., 2007; Harikumar et al., 2008; Lust et al., 2005; Monteghirfo et al., 2008), prostate cancer (Colgate et al., 2007; Delmulle et al., 2008), and hepatoma cell lines (Ho et al., 2008). As an additional antiproliferative mechanism, XN was identified as an inducer of cellular differentiation in a human leukemia cell line (Gerhauser et al., 2002a). Induction of differentiation and apoptosis was also observed in preadipocytes (Mendes et al., 2008; Yang et al., 2007, 2008).

Unfortunately, concentrations of XN in beer are very low (about 0.1 mg/L), due to

its cyclization to the corresponding isomeric prenylflavanone isoxanthohumol (IX) during brewing (Gerhauser et al., 2002a). IX represents the major prenylflavonoid in beer (Fig. 1; reviewed in Gerhauser, 2005a).

ANTI-ANGIOGENIC POTENTIAL OF XN

Since the groundbreaking work of Judah Folkman in the 1970s, angiogenesis is recognized as a key mechanism in tumorigenesis and formation of metastases (Folkman, 1971). Without adequate blood supply, a tumour cannot grow beyond a critical size of 1-2 mm due to a lack in nutrients and oxygen (Carmeliet and Jain, 2000). Angiogenesis describes the formation of new blood vessels from pre-existing microvasculature. It is generally initialized by release of angiogenic factors from the tumour, degradation of the basement membrane by lytic enzymes, endothelial cell proliferation, migration and tube formation (differentiation), and finally lumen formation and stabilization of the new vessel with a basement membrane and pericytes (Wernert et al., 1999). To investigate anti-angiogenic properties of XN, IX, and a series of additional promising cancer chemopreventive compounds, we established a human *in vitro* anti-angiogenic test system based on the principle of wound healing (Bertl et al., 2004). Fragments of placental veins or arteries were cultured in the presence or absence of the compounds in fibrin gels for 3 weeks. Resveratrol from red wine was used as a positive control substance and demonstrated about 50% inhibition at 1 and 10 μM concentrations. XN and IX were even more potent and inhibited microcapillary growth by 19.1% and 89.8% (XN) and 63.1% and 86.5% (IX) at 1 and 10 μM concentrations, respectively (Fig. 4). The IC_{50} value of XN was determined as 2.2 μM (data not shown; from Bertl, 2005).

We used Raw 264.7 murine macrophages stimulated with lipopolysaccharides (LPS) and human microvascular endothelial cells (HMEC-1) under hypoxic conditions as *in vitro* models to investigate the effects of XN on the release of pro-angiogenic factors at the transcriptional level by RT-PCR. mRNA expression of pro-angiogenic signaling molecules, expression and activity of proteolytic enzymes (matrix metalloproteinases) and effects on endothelial cell functions, including proliferation, migration and capillary formation were determined as described (Bertl et al., 2006). Cells were treated with test compounds in a time (0-24h)- and concentration-dependent (0.2-25 μM) manner. In both cell types, XN potently inhibited mRNA expression of proangiogenic factors, including but not limited to VEGF (vascular endothelial growth factor), cyclooxygenase 2, iNOS and the protooncogen c-Myc (Bertl, 2005).

Raw macrophages secrete matrix metalloproteinases MMP-9 and -2 as well as their tissue inhibitors TIMP-1 and -2 upon stimulation with LPS. RT-PCR experiments revealed that XN delayed and strongly suppressed maximal MMP-9 mRNA expression at nanomolar concentrations. Interestingly, TIMP-1 and -2 mRNA expression was induced transiently with a maximum at 2-4h after LPS-stimulation, and the influence of XN was less pronounced. Using gelatine zymography we could demonstrate that XN treatment strongly suppressed MMP-9 activity, which is predominantly expressed in Raw macrophages, in a time- and concentration-dependent manner (Bertl, 2005, and in preparation). XN did not inhibit MMP-2 activity in human umbilical vein endothelial cells (HUVEC), nor did it inhibit MMP-2 and MMP-9 activity in Kaposi sarcoma cells KS-IMM (Albini et al., 2006).

In addition, XN was studied extensively for effects on endothelial cells. Halfmaximal inhibition of HMEC-1 proliferation was observed at $6.3 \pm 0.8 \mu\text{M}$ after 48h of incubation. To assess endothelial cell migration, HMEC-1 were grown to confluence and then scraped with a pipette tip. Wound closure in the absence or presence of 0.001 to 10 μM XN after an incubation time of 18h was monitored microscopically and evaluated by digital image analysis. XN demonstrated extremely potent inhibition of HMEC-1 migration with an IC_{50} value of $0.03 \pm 0.007 \mu\text{M}$. HMEC-1 seeded on Matrigel™ form capillary-like tubes after an incubation period of 6h. XN was added at concentrations up to 10 μM . The influence on tube formation was assessed visually. XN potently inhibited the formation of tubule-like structures at 1, 5 and 10 μM concentrations, whereas at

0.1 μM , some tubes started to form (reviewed in Gerhauser, 2005a). Similar effects were reported by Albini (Albini et al., 2006).

ANTI-ESTROGENIC ACTIVITIES OF XN

Hormones like estrogen (17 β -estradiol, E2) play an important role in carcinogenesis and are regarded as endogenous tumour promoters (Yager and Davidson, 2006). Effects of estrogens are mediated through interaction with estrogen receptors ER α and ER β , which act as hormone-dependent nuclear transcription factors. Estradiol enters the cell by passive diffusion and binds to the estrogen receptor, which is located in the cytosol. Ligand binding leads to receptor dimerization and translocation into the nucleus. There, the receptor dimer binds to the so called estrogen-responsive element in the promoter region of estrogen-responsive genes. DNA binding also recruits a series of coregulators (activators or repressors) that facilitate or inhibit gene transcription (Griekspoor et al., 2007). Activation of estrogen signalling results in novel protein synthesis and in enhanced cell proliferation. In addition, recent reports indicate that E2 or its metabolites also might be involved in tumour initiation and act as mutagens that contribute to the formation of DNA adducts and chromosomal changes (Cavaliere et al., 2006). Plant derived estrogen-like compounds, so-called phytoestrogens, share structural similarities with E2, in particular the aromatic ring system and distance of hydroxyl groups, which allows binding to estrogen receptors and provoke SERM (selective estrogen receptor modulator)-like activities (Vollmer and Zierau, 2004).

Hops have repeatedly been reported to possess estrogenic properties. Therefore, we investigated pro- and anti-estrogenic properties of XN and IX in Ishikawa cell culture. This cell line derived from a human endometrial cancer responds to estrogens with elevated alkaline phosphatase (ALP) activity. Concomitant treatment with estrogens and test compounds allows the identification of anti-estrogens. XN efficiently inhibited estrogen-mediated induction of alkaline phosphatase without possessing intrinsic estrogenic potential. On the other hand, IX was identified as a weak estrogen agonist. Currently, we cannot exclude that these effects are mediated by partial demethylation to form 8-prenylnaringenin, which was about 1000-fold more potent than IX (Gerhauser et al., 2002a). In MCF-7 human breast cancer cells, estradiol treatment reduced ALP expression and activity. These effects were reversible by co-treatment with a pure antiestrogen. XN reduced expression of intestinal ALP, but not of tissue non-specific ALP expression, and reduced ALP activity, without exerting an estrogen-like cell growth promoting effect (Guerreiro et al., 2007).

Interestingly, XN may also reduce the generation of estrogens by inhibiting the enzymatic activity of aromatase, which converts testosterone to estrogen. Using a human recombinant enzyme source, XN dose-dependently inhibited aromatase activity with an IC₅₀ value of 2.5 μM , whereas IX was less potent with an IC₅₀ value of 8.6 μM (Fig. 4). Inhibition of aromatase activity by XN has been described previously (Monteiro et al., 2006).

The anti-estrogenic effect of XN was confirmed in vivo. Prepubertal (19 days old) female Sprague-Dawley rats (n = 6 per treatment) received XN (100 mg/kg body weight (bw), either by gavage suspended in 2% starch suspension, or intraperitoneally (i.p.) and subcutaneously (s.c.), dissolved in corn oil, respectively) on three consecutive days (two groups for each treatment). One of each treatment groups was injected simultaneously with 1 $\mu\text{g}/\text{kg}$ bw 17- β -ethinylestradiol (EE) s.c. every day. As positive and negative controls, two groups were treated with either solvent or EE (1 $\mu\text{g}/\text{kg}$ bw, s.c.), respectively. Body weight changes were monitored daily. On day four, rats were sacrificed by CO₂ and uteri were weighed. Uteri weights were calculated in relation to body weights. Normalized uterine weights were calculated as a percentage of the untreated control to assess estrogenic effects. The EE-treated group was set as 100%. XN reduced the EE-induced gain in uterine wet weight by 31%, without any stimulatory effect in the absence of EE (reviewed in Gerhauser, 2005a). Quantitative analyses of mRNA expression of estrogen-dependent target genes in the uteri confirmed anti-

estrogenic potential of XN. Complement 3, GAPDH and insulin-like growth factor-1 (IGF-1) mRNA expression in uteri of prepubertal rats (as described above) was quantified by real time RT-PCR as described previously (Diel et al., 2000) with minor modifications. Cytochrome C oxidase expression was used for normalization. mRNA expression of all three genes was induced by EE application. Concomitant XN treatment significantly inhibited EE-induced expression by about 30-60%, although uterine weights were less affected in this follow-up experiment (Strathmann et al., in preparation).

To address the question whether the anti-estrogenic effects of XN are indeed due to ER binding, we performed binding analyses using a fluorescence polarization method (Fokialakis et al., 2004). Relative binding affinities of XN to ER α and ER β were determined as 0.08 and 0.11 in relation to E2 set as 100. Interestingly, ER docking calculations superimposing the global minimum structure of XN to an ER α -raloxifene complex show similarities between XN and raloxifene-binding to the ligand-binding cavity of ER α . This model indicates that XN may exert anti-estrogenic potential by modulating the 3D protein structure of ER and consequently alter binding of coregulators. Further studies have to confirm this hypothesis (Strathmann et al., in preparation).

Since estrogens also have important regulatory functions during development, for safety issues we investigated the influence of XN on rat reproduction. In two fertility studies, XN (100 mg/kg bw/day), given either for four weeks prior to or during mating, gestation and nursing, did not cause any adverse effect on female reproduction and the development of offspring. Lifelong treatment at a daily dose of 100 mg/kg bw (in drinking water) in a two-generation study also did not affect the development of SD rats. Noteworthy, treatment of male rats prior to mating significantly ($p = 0.027$) increased the sex ratio of male to female offspring (Hussong et al., 2005).

FURTHER INVESTIGATIONS ON XN

Safety after long-term application is a central issue for compounds intended for the prevention of chronic diseases. When XN was applied by gavage for four weeks at a daily dose of 1000 mg/kg bw per day to female SD rats, we observed a 30% reduction in liver weights, which was interpreted as weak hepatic toxicity. Other organs were not affected. At a 10-fold lower concentration in drinking water, xanthohumol did not show any toxic effects (Hussong et al., 2005). Interestingly, XN also reduced liver weights and white adipose tissue in a diabetic mouse model. This was associated with an amelioration of lipid and glucose metabolism (Nozawa, 2005).

Bioavailability, pharmacokinetics, and metabolisms are important factors which influence in vivo efficacy of chemopreventive agents. We investigated the oral bioavailability of XN in a kinetic experiment. XN was applied to female SD rats at a single dose of 1000 mg/kg bw. XN-4'-O-glucuronide was identified as the major metabolite with a maximum plasma concentration of 3.1 μ M 4h after application, whereas unmetabolized XN was detectable with a maximum concentration 0.34 μ M (equal to 0.12 μ g/ml) 4h post-administration. About 90% of the applied XN was recovered unmetabolized from feces after 24 and 48h. This was confirmed in studies using 14 C-labelled XN (Berwanger et al., 2005). Recovery of radioactivity in organs was very low and mainly limited to the gastrointestinal tract (data not shown). Sixteen novel metabolites were isolated from fecal samples in addition to six previously known metabolites (Nookandeh et al., 2004). Although anti-infective activities of XN have been reported (reviewed in Gerhauser, 2005b), XN did not influence the composition of fecal microbiota after application of 100 mg XN/kg bw daily for 4 weeks (Hanske et al., 2005).

Based on the expected high concentrations of XN in the GI tract, we investigated colon cancer preventive effects of XN, using azoxymethane-induced preneoplastic lesions in rat colon as a model. Unfortunately, treatment with XN for 9 weeks at 100 mg/kg bw/day in drinking water did not lower the total number of aberrant crypt foci (ACF) or the number of aberrant crypts/ACF (Michel, 2005).

BREAST CANCER PREVENTIVE EFFICACY

As a first indication of breast cancer chemopreventive efficacy, XN was tested *ex vivo* in the mouse mammary gland organ culture (MMOC) model. Preneoplastic mammary lesion formation is induced by dimethylbenz[*a*]anthracene (DMBA) treatment under growth factor-controlled conditions. Notably, XN dose-dependently prevented lesion formation at 200-fold lower concentrations than resveratrol, with an IC₅₀ value of 0.02 μ M (Gerhauser et al., 2002a).

Novel data indicate that XN prevents DMBA-induced mammary carcinogenesis in rats. Female SD rats were treated with 0, 10 and 100 mg XN/kg bw in drinking water for 16 weeks. After an initial pretreatment for 9 days all animals were gavaged with a single dose of DMBA (100 mg/kg bw at the age of 51 days). Rats were weighed and received freshly prepared XN-solutions every second day. After 16 weeks of intervention, rats were sacrificed; tumors were isolated, weighed and fixed for pathological examination. Tumour incidence was not significantly influenced by either XN treatment (water 71%, XN-10 47%, XN-100 64%). However, XN treatment at the high dose significantly reduced tumour multiplicity and tumour burden. Also, treatment with XN-100 resulted in a significantly higher bodyweight ($p < 0.05$) at the end of the study, compared to the XN-10 and DMBA treatment groups. This was associated with a faster recovery from weight loss after DMBA application and suggested that the animals' overall condition was positively affected by XN. XN was not protective when applied only during the promotion phase after carcinogen treatment (Strathmann et al., in preparation).

In an additional study, intravital microscopy was used as an *in vivo* model to characterise the effect of XN on tumour growth and angiogenesis with high temporal and spatial resolution. Fourteen female adult severe combined immune deficient (SCID) mice were fitted with a dorsal skinfold chamber as described before (Sckell and Leunig, 1999). Tumor fragments (1 mm³) of MX-1 estrogen-receptor negative human breast cancer xenografts were implanted into the dorsal skinfold chamber and observed weekly by intravital microscopy (Klenke et al., 2005). Starting from day 15 after tumour implantation, groups of 7 mice were treated with XN (1000 mg/kg bw/day in DMSO, applied subcutaneously) or solvent, respectively, for 7 and 14 days. Two-dimensional tumour surface was documented with digital photography using bright field microscopy. Functional vessel density as an indicator of angiogenesis was quantified using intravital fluorescence video microscopy after injection of FITC-labelled dextran as a plasma marker. In this animal model, s.c. application of XN for 7 and 14 days inhibited the growth of the breast tumour xenografts by 46% and 83%, respectively, in comparison with the untreated control group, and reduced the size of established tumours by 30 and 56%, respectively. Concomitantly, XN-treatment for 14 days reduced tumour-induced neovascularization by 33% in comparison with the untreated control group (reviewed in Gerhauser, 2005a; Bertl et al., in preparation). A reduction of tumour xenograft growth and inhibition of angiogenesis by XN was also reported by Albini and Monteiro (Albini et al., 2006; Monteiro et al., 2008).

CONCLUSIONS

In conclusion, we have identified XN as a broad-spectrum chemopreventive agent. We have demonstrated that XN prevents carcinogen-induced mammary carcinogenesis in rats. Multiple mechanisms, including modulation of DMBA metabolism, anti-estrogenic, anti-angiogenic and anti-proliferative activity may account for these effects and are under further investigation. In addition, XN also potently inhibited MX-1 tumour xenotransplant growth and neovascularization in SCID mice. A scheme summarizing how XN prevents hormone-dependent tumorigenesis is given in Figure 5.

Literature Cited

Albini, A., Dell'eva, R., Vene, R., Ferrari, N., Buhler, D.R., Noonan, D.M. and Fassina, G. 2006. Mechanisms of the antiangiogenic activity by the hop flavonoid xanthohumol: NF- κ B and Akt as targets. *FASEB J.* 20:527-529.

- Bertl, E., Klimo, K., Heiss, E., Klenke, F., Peschke, P., Becker, H., Eicher, T., Herhaus, C., Kapadia, G., Bartsch, H. and Gerhauser, C. 2004. Identification of novel inhibitors of angiogenesis using a human in vitro anti-angiogenesis assay. *Int. J. Cancer Prev.* 1:47-61.
- Bertl, E. 2005. Inhibition of angiogenesis by potential cancer chemopreventive agents – Establishment of a human in vitro anti-angiogenesis assay and mechanistic evaluation of potent inhibitors. Ph.D. Thesis, University of Heidelberg.
- Bertl, E., Bartsch, H. and Gerhauser, C. 2006. Inhibition of angiogenesis and endothelial cell functions are novel sulforaphane-mediated mechanisms in chemoprevention. *Mol. Cancer Ther.* 5:575-585.
- Berwanger, S., Frank, N., Knauff, J. and Becker, H. 2005. Biosynthetic (14)C-labelling of xanthohumol in hop cones. *Mol. Nutr. Food Res.* 49:857-860.
- Carmeliet, P. and Jain, R.K. 2000. Angiogenesis in cancer and other diseases. *Nature* 407:249-257.
- Cavalieri, E., Chakravarti, D., Guttenplan, J., Hart, E., Ingle, J., Jankowiak, R., Muti, P., Rogan, E., Russo, J., Santen, R. and Sutter, T. 2006. Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention. *Biochim. Biophys. Acta.* 1766:63-78.
- Cho, Y.C., Kim, H.J., Kim, Y.J., Lee, K.Y., Choi, H.J., Lee, I.S. and Kang, B.Y. 2008. Differential anti-inflammatory pathway by xanthohumol in IFN- γ and LPS-activated macrophages. *Int. Immunopharmacol.* 8:567-573.
- Colgate, E.C., Miranda, C.L., Stevens, J.F., Bray, T.M. and Ho, E. 2007. Xanthohumol, a prenylflavonoid derived from hops induces apoptosis and inhibits NF- κ B activation in prostate epithelial cells. *Cancer Lett.* 246:201-209.
- De Flora, S. and Ferguson, L.R. 2005. Overview of mechanisms of cancer chemopreventive agents. *Mutat. Res.* 591:8-15.
- Dell'Eva, R., Ambrosini, C., Vannini, N., Piaggio, G., Albini, A. and Ferrari, N. 2007. AKT/NF-kappaB inhibitor xanthohumol targets cell growth and angiogenesis in hematologic malignancies. *Cancer* 110:2007-2011.
- Delmulle, L., Berghe, T.V., Keukeleire, D.D. and Vandenabeele, P. 2008. Treatment of PC-3 and DU145 prostate cancer cells by prenylflavonoids from hop (*Humulus lupulus* L.) induces a caspase-independent form of cell death. *Phytother. Res.* 22:197-203.
- Diel, P., Schulz, T., Smolnikar, K., Strunck, E., Vollmer, G. and Michna, H. 2000. Ability of xeno- and phytoestrogens to modulate expression of estrogen-sensitive genes in rat uterus: estrogenicity profiles and uterotrophic activity. *J. Steroid Biochem. Mol. Biol.* 73:1-10.
- Fokialakis, N., Lambrinidis, G., Mitsiou, D.J., Aligiannis, N., Mitakou, S., Skaltsounis, A.L., Pratsinis, H., Mikros, E. and Alexis, M.N. 2004. A new class of phytoestrogens; evaluation of the estrogenic activity of deoxybenzoins. *Chem. Biol.* 11:397-406.
- Folkman, J. 1971. Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* 285:1182-1186.
- Gerhauser, C., Alt, A., Heiss, E., Gamal-Eldeen, A., Klimo, K., Knauff, J., Neumann, I., Scherf, H.R., Frank, N., Bartsch, H. and Becker, H. 2002a. Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop. *Mol. Cancer Ther.* 1:959-969.
- Gerhauser, C., Alt, A.P., Klimo, K., Knauff, J., Frank, N. and Becker, H. 2002b. Isolation and potential cancer chemopreventive activities of phenolic compounds of beer. *Phytochem. Rev.* 1:369-377.
- Gerhauser, C., Klimo, K., Heiss, E., Neumann, I., Gamal-Eldeen, A., Knauff, J., Liu, G.Y., Sitthimonchai, S. and Frank, N. 2003. Mechanism-based in vitro screening of potential cancer chemopreventive agents. *Mutat. Res.* 523-524:163-172.
- Gerhauser, C. 2005a. Beer constituents as potential cancer chemopreventive agents. *Eur. J. Cancer* 41:1941-1954.
- Gerhauser, C. 2005b. Broad spectrum anti-infective potential of xanthohumol from hop

- (*Humulus lupulus* L.) in comparison with activities of other hop constituents and xanthohumol metabolites. *Mol. Nutr. Food Res.* 49:827-831.
- Griekspoor, A., Zwart, W., Neefjes, J. and Michalides, R. 2007. Visualizing the action of steroid hormone receptors in living cells. *Nucl. Recept. Signal.* 5:e003.
- Guerreiro, S., Monteiro, R., Martins, M.J., Calhau, C., Azevedo, I. and Soares, R. 2007. Distinct modulation of alkaline phosphatase isoenzymes by 17 β -estradiol and xanthohumol in breast cancer MCF-7 cells. *Clin. Biochem.* 40:268-273.
- Hanske, L., Hussong, R., Frank, N., Gerhauser, C., Blaut, M. and Braune, A. 2005. Xanthohumol does not affect the composition of rat intestinal microbiota. *Mol. Nutr. Food Res.* 49:868-873.
- Harikumar, K.B., Kunnumakkara, A.B., Ahn, K.S., Anand, P., Krishnan, S., Guha, S. and Aggarwal, B.B. 2008. Modification of the cysteine residues in I κ B α kinase and NF- κ B (p65) by xanthohumol leads to suppression of NF- κ B-regulated gene products and potentiation of apoptosis in leukemia cells. *Blood* (doi:10.1182/blood-2008-04-151944).
- Ho, Y.C., Liu, C.H., Chen, C.N., Duan, K.J. and Lin, M.T. 2008. Inhibitory effects of xanthohumol from hops (*Humulus lupulus* L.) on human hepatocellular carcinoma cell lines. *Phytother. Res.* 22:1465-1468.
- Hussong, R., Frank, N., Knauff, J., Itrich, C., Owen, R., Becker, H. and Gerhauser, C. 2005. A safety study of oral xanthohumol administration and its influence on fertility in Sprague Dawley rats. *Mol. Nutr. Food Res.* 49:861-867.
- Kac, J., Plazar, J., Mlinaric, A., Zegura, B., Lah, T.T. and Filipic, M. 2008. Antimutagenicity of hops (*Humulus lupulus* L.): bioassay-directed fractionation and isolation of xanthohumol. *Phytomedicine* 15:216-220.
- Khupse, R.S. and Erhardt, P.W. 2007. Total synthesis of xanthohumol. *J. Nat. Prod.* 70:1507-1509.
- Klenke, F.M., Merkle, T., Fellenberg, J., Abdollahi, A., Huber, P.E., Gebhard, M.M., Ewerbeck, V. and Sckell, A. 2005. A novel model for the investigation of orthotopically growing primary and secondary bone tumours using intravital microscopy. *Lab. Anim.* 39:377-383.
- Lee, S.H., Kim, H.J., Lee, J.S., Lee, I.S. and Kang, B.Y. 2007. Inhibition of topoisomerase I activity and efflux drug transporters' expression by xanthohumol from hops. *Arch. Pharm. Res.* 30:1435-1439.
- Lust, S., Vanhoecke, B., Janssens, A., Philippe, J., Bracke, M. and Offner, F. 2005. Xanthohumol kills B-chronic lymphocytic leukemia cells by an apoptotic mechanism. *Mol. Nutr. Food Res.* 49:844-850.
- Mendes, V., Monteiro, R., Pestana, D., Teixeira, D., Calhau, C. and Azevedo, I. 2008. Xanthohumol influences preadipocyte differentiation: implication of antiproliferative and apoptotic effects. *J. Agric. Food Chem.* 56:11631-11637.
- Michel, S. 2005. Azoxymethane induced colon carcinogenesis in rats: influence of xanthohumol on early biomarkers for chemoprevention. Diploma thesis, University of Hohenheim.
- Monteghirfo, S., Tosetti, F., Ambrosini, C., Stigliani, S., Pozzi, S., Frassoni, F., Fassina, G., Soverini, S., Albini, A. and Ferrari, N. 2008. Antileukemia effects of xanthohumol in Bcr/Abl-transformed cells involve nuclear factor- κ B and p53 modulation. *Mol. Cancer Ther.* 7:2692-2702.
- Monteiro, R., Becker, H., Azevedo, I. and Calhau, C. 2006. Effect of hop (*Humulus lupulus* L.) flavonoids on aromatase (estrogen synthase) activity. *J. Agric. Food Chem.* 54:2938-2943.
- Monteiro, R., Calhau, C., Silva, A.O., Pinheiro-Silva, S., Guerreiro, S., Gartner, F., Azevedo, I. and Soares, R. 2008. Xanthohumol inhibits inflammatory factor production and angiogenesis in breast cancer xenografts. *J. Cell. Biochem.* 104:1699-1707.
- Monteiro, R., Faria, A., Azevedo, I. and Calhau, C. 2007. Modulation of breast cancer cell survival by aromatase inhibiting hop (*Humulus lupulus* L.) flavonoids. *J. Steroid*

Biochem. Mol. Biol. 105:124-130.

- Nookandeh, A., Frank, N., Steiner, F., Ellinger, R., Schneider, B., Gerhauser, C. and Becker, H. 2004. Xanthohumol metabolites in faeces of rats. *Phytochemistry* 65:561-570.
- Nozawa, H. 2005. Xanthohumol, the chalcone from beer hops (*Humulus lupulus* L.), is the ligand for farnesoid X receptor and ameliorates lipid and glucose metabolism in KK-A(y) mice. *Biochem. Biophys. Res. Commun.* 336:754-761.
- Pan, L., Becker, H. and Gerhauser, C. 2005. Xanthohumol induces apoptosis in cultured 40-16 human colon cancer cells by activation of the death receptor- and mitochondrial pathway. *Mol. Nutr. Food Res.* 49:837-843.
- Plazar, J., Filipic, M. and Groothuis, G.M. 2008. Antigenotoxic effect of Xanthohumol in rat liver slices. *Toxicol. In Vitro* 22:318-327.
- Plazar, J., Zegura, B., Lah, T.T. and Filipic, M. 2007. Protective effects of xanthohumol against the genotoxicity of benzo(a)pyrene (BaP), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and tert-butyl hydroperoxide (t-BOOH) in HepG2 human hepatoma cells. *Mutat. Res.* 632:1-8.
- Sckell, A. and Leunig, M. 1999. Dorsal skinfold chamber preparation in mice. *Meth. Mol. Med.* 46:95-105.
- Sporn, M.B. and Newton, D.L. 1979. Chemoprevention of cancer with retinoids. *Fed. Proc.* 38:2528-2534.
- Stresser, D.M., Turner, S.D., McNamara, J., Stocker, P., Miller, V.P., Crespi, C.L. and Patten, C.J. 2000. A high-throughput screen to identify inhibitors of aromatase (CYP19). *Anal. Biochem.* 284:427-430.
- Vogel, S. and Heilmann, J. 2008. Synthesis, cytotoxicity, and antioxidative activity of minor prenylated chalcones from *Humulus lupulus*. *J. Nat. Prod.* 71:1237-1241.
- Vogel, S., Ohmayer, S., Brunner, G. and Heilmann, J. 2008. Natural and non-natural prenylated chalcones: synthesis, cytotoxicity and anti-oxidative activity. *Bioorg. Med. Chem.* 16:4286-4293.
- Vollmer, G. and Zierau, O. 2004. [What are phytoestrogens and phyto-SERMS]. *Pharm. Unserer Zeit* 33:378-383.
- Wernert, N., Stanjek, A., Kiriakidis, S., Hugel, A., Jha, H.C., Mazitschek, R. and Giannis, A. 1999. Inhibition of angiogenesis in vivo by ets-1 antisense oligonucleotides-inhibition of Ets-1 transcription factor expression by the antibiotic fumagillin. *Angew. Chem. Int. Ed. Engl.* 38:3228-3231.
- Yager, J.D. and Davidson, N.E. 2006. Estrogen carcinogenesis in breast cancer. *N. Engl. J. Med.* 354:270-282.
- Yang, J.Y., Della-Fera, M.A., Rayalam, S. and Baile, C.A. 2007. Effect of xanthohumol and isoxanthohumol on 3T3-L1 cell apoptosis and adipogenesis. *Apoptosis* 12:1953-1963.
- Yang, J.Y., Della-Fera, M.A., Rayalam, S. and Baile, C.A. 2008. Enhanced effects of xanthohumol plus honokiol on apoptosis in 3T3-L1 adipocytes. *Obesity (Silver Spring)*. 16:1232-1238.