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Critical role of pro-apoptotic Bcl-2 family members in andrographolide-induced apoptosis in human cancer cells

J. Zhou, S. Zhang, C.N. Ong, H.M. Shen. *National University of Singapore, Department of Community, Occupational and Family Medicine, Singapore, Singapore*

Background: Andrographolide (Andro), a diterpenoid lactone isolated from a traditional herbal medicine *Andrographis paniculata*, is known to possess a potent anti-inflammatory activity. In this study, we attempted to investigate the anti-cancer potential of Andro by examining Andro-induced apoptotic cell death and the underlying molecular mechanisms.
Material and Methods: We utilized DAPI staining and DNA content analysis to detected apoptotic cell death; immunoprecipitation, western blot and immunofluorescence were used to determine the involvement of caspase cascade and some Bcl-2 family members; transient transfection and siRNA technology were used to confirm the protein functions in Andro-induced apoptosis.
Results: First, we found that Andro-induced apoptotic cell death in various human cancer cells. Next, we examined the apoptotic signaling pathway elicited by Andro. It was found that Andro is capable of activating the initiator caspases for the extrinsic death receptor pathway and mitochondrial pathway, respectively. Various caspase inhibitors could effectively prevent Andro-induced cell death. We further investigated the role of Bcl-2 family members to understand the regulatory mechanisms in Andro-induced apoptosis. Andro treatment triggered a caspase-8 dependent Bid cleavage, followed by a series of sequential events including Bax conformational change and mitochondrial translocation, release of cytochrome c from mitochondria and activation of effector caspase 3. Selective inhibition of caspase 8 activity blocked Bid cleavage, conformational change of Bax and Andro-induced apoptosis. Consistently, knockdown of Bid protein using siRNA technique suppressed Andro-induced Bax conformational change and apoptosis.
Conclusions: Data from this study provide convincing evidence that Andro is capable of inducing apoptosis in human cancer cells, and the pro-apoptotic Bcl-2 family members (Bid and Bax) are the key mediators in relaying the cell death signaling initiated by Andro from caspase 8 to mitochondria and then to downstream effector caspases, and eventually leading to apoptotic cell death.

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Green tea extracts inhibits HGF-induced HNSCC progression in vitro

K. Chul-Ho[1], L. Jae-Ho[2], L. HaeJin[1]. *[1]Ajou University, Otolaryngology, Suwon, Korea; [2]Ajou University, Biochemisty, Suwon, Korea*

Purpose: Aberrant activation of hepatocyte growth factor(HGF) and its receptor, c-Met, has been known to be involved in many human cancer development and progression. During the search for an effective molecule inhibitor of HGF/c-Met signaling, we have found that Epigallocatechin-3-gallate(EGCG), the major bioactive polyphenol present in green tea, might inhibit HGF/c-Met signaling. Studies were performed to address whether EGCG inhibit HGF-dependent tumor proliferation and invasion in HNSCC.
Method: We performed RT-PCR and Western blot of HNSCC cell line. Proliferation assay, dispersion assay, wound healing assay, and invasion assay were performed in HGF 0, 10, 30 ng/mL HGF10+EGCG 1 μM, HGF10+EGCG10 μM, HGF30+EGCG1 μM, HGF30+EGCG10 μM. RT-PCR and zymography were performed to examine the roles of MMP-2 and MMP-9, as well as the relationship between HGF and MMPs in FaDu invasiveness. In addition, we confirmed HGF-mediated plasmin activation.
Results: Exogenous HGF significantly enhanced the growth of HNSCC cell and this phenomenon was inhibited by EGCG in dose-dependant manner ($p < 0.05$). EGCG inhibited HGF-induced scattering of HNSCC cell. EGCG inhibited HGF-mediated migration and invasion of HNSCC cell in dose-dependent ($p < 0.05$). EGCG inhibits the HGF-Met-uPA-Plasmin network and MMP2, 9.
Conclusions: Inhibition of HGF/Met signaling by EGCG leads to decrease of proliferation and invasion in vitro, suggesting the possible use of EGCG in HNSCC associated with downregulation of HGF/Met signaling and the HGF-Met-uPA-Plasmin network and MMP2, 9.

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Post-initiation induction of NQO1 inhibits colon carcinogenesis in Sprague-Dawley rats

A. Begleiter[1,2,3], K. Sivananthan[1], G.M. Lefas[1], A.W. Maksymiuk[2], R.P. Bird[4]. *[1]CancerCare Manitoba, Manitoba Institute of Cell Biology, Winnipeg, Canada; [2]University of Manitoba, Department of Internal Medicine, Winnipeg, Canada; [3]University of Manitoba, Department of Pharmacology & Therapeutics, Winnipeg, Canada; [4]University of Waterloo, Department of Gerontology, Waterloo, Canada*

Background: Phase II detoxifying enzymes may play a significant role in preventing carcinogen induced colon cancer at the initiation and post-initiation stage, but it is not clear that NAD(P)H:quinone oxidoreductase 1 (NQO1) contributes to this effect. We showed that dietary oltipraz selectively induces NQO1 in the colon of Sprague-Dawley rats without increasing the levels of other phase II enzymes in these animals. Using this model, we demonstrated that induction of NQO1 by oltipraz prior to administration of carcinogens decreases the formation of preneoplastic lesions called aberrant crypt foci (ACF) in the colons of these rats. These results provided the first direct evidence that induction of NQO1 alone, without induction of other phase II detoxifying enzymes, can inhibit initiation of colon carcinogenesis, suggesting that this enzyme plays an important role in inhibiting carcinogen induced colon cancer. In this study we used the same rat model to investigate if post-initiation induction of NQO1 can inhibit colon carcinogenesis. In addition, we examined the effect of post-initiation induction of NQO1 on apoptosis in cells in ACF as a possible mechanism for the inhibition of carcinogenesis.
Materials and Methods: Sprague-Dawley rats were treated with the colon carcinogen, azoxymethane (AOM), and then were fed either control diet or diet containing 200 ppm oltipraz. The number of ACF at 12 weeks and the number of adenomas and tumors at 29 weeks in the colons of the rats were enumerated and the two treatment groups were compared. Paraffin blocks were prepared from colon sections obtained at 12 weeks following AOM treatment, slices were stained with hematoxylin and eosin, the percentage of apoptotic cells in ACF were enumerated, and oltipraz and control groups were compared.
Results: Rats fed oltipraz containing diet following treatment with AOM had 60% fewer ACF after 12 weeks compared with rats fed a control diet. Similarly, rats fed oltipraz containing diet after AOM treatment developed 40% fewer colon adenomas and fewer colon tumors than rats fed a control diet. There was also a 60% increase in the percentage of apoptotic cells in ACF from oltipraz fed rats compared with ACF from control fed rats.
Conclusions: These results provide strong evidence that NQO1 can contribute to inhibition of colon carcinogenesis at the post-initiation stage. A possible mechanism for this effect may be that induction of NQO1 results in increased apoptosis in carcinogen initiated colonic epithelial cells that prevents these cells from progressing to a neoplastic state.
Supported by grants from the Canadian Institutes of Health Research and CancerCare Manitoba Foundation.

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In vitro and in vivo anti-tumor activities of SG135 in prostate cancer

H.O. Yang[1], E.M. Yang[1], S.N. Kim[1], J.H. Yoo[1], Y.K. Kim[2]. *[1]Korea Institute of Science and Technology, Gangneung Institute, Gangneung, Korea; [2]Kwandong University, Department of Pharmacology, Gangneung, Korea*

Considerable attention has recently been focused on dietary or medicinal phytochemicals that possess cancer chemopreventive or tumor growth inhibitory properties. *Panax ginseng* C.A. Meyer has long been used in traditional oriental medicine. According to recent epidemiologic studies conducted in Korea, ginseng consumption reduced the risk of cancers of stomach, esophagus, colon, and lung. A wide array of ginsenosides have been purified and many of them have been tested for their anti-carcinogenic potential.
Heat treatment of ginseng at a temperature higher than that applied to the conventional preparation of red ginseng enhanced the yield of Rg_3 and Rg_5, which are two of the red ginseng specific saponins, accounting for 39% and 19% of all ginsenosides, respectively. And another ginsenosides, Rk1, Rk2, Rk3, Rs4, Rs5, Rs6 and Rs7 were also contained in heat processed ginseng (it is called Sun Ginseng, SG). *In vitro* anti-tumor promoting, chemopreventive and antioxidant activities of heat processed ginseng (SG) were reported.
SG135, saponin-rich fraction, was processed from SG and contained high amount of Rk1, Rg_3 and Rg_5 ginsenosides. The present study was performed to evaluate *in vitro* and *in vivo* anti-tumor effects of SG135 using DU 145 prostate cancer cell line.
The IC_{50} value of SG135 was 20.0 ± 3.7 ug/ml. The treatment with SG135, 130 ug/ml, induced the early stage of apoptosis by 5.7-fold in DU 145 cells using annexin V^+/PI staining.