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

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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

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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

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

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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.

In vivo study was performed in BALB/c nude mice. Six weeks old nude mice were divided 4 groups, 10 mice of each group, control, SG135, 5 mg/kg treated, SG135, 20 mg/kg treated and SG135 60 mg/kg treated groups. For the mice of control group were fed saline through out the experimental period. For the mice of three kinds of SG135 treated groups were fed SG at the dose of 5, 20 and 60 mg/kg/day, p.o. three times a week. After medication of SG135 for 12 days, tumor cells, 1×10^7 cells/mouse, were inoculated by s.c. on the flank of mouse. The tumor sizes were measured twice a week. The tumor growth was inhibited in all SG135, 5, 20 and 60 mg/kg/day treated groups, 21.0%, 26.0% and 25.0%, respectively, on day 49 after tumor inoculation when compared with control group. The survival rate were prominently increased in the mice of SG 20 and 60 mg/kg/day treated groups, 150.0% and 200.0%, respectively, on day 120 after tumor inoculation when compared with control group. These data presented that SG135 treatment was most effective in tumor growth inhibition and prominently effective in increase of average survival rate.

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Down-regulation of Sphingosine 1-Phosphate Receptor-1 in intestinal tumorigenesis

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Introduction: The bioactive sphingolipid, Sphingosine 1-Phosphate (S1P) is implicated in the regulation of cellular proliferation, migration, and survival via its G protein-coupled receptors S1P1–5. While animal models of intestinal neoplasia have demonstrated a beneficial effect of dietary sphingolipids in chemoprevention, the role of S1P in colon cancer is still unclear. The purpose of this study was to help define the role of the S1P receptor-ligand system in colon cancer.
Methods: Small intestine specimens of bigenic S1P1+/−Apcmin and S1P1+/+Apcmin mice were compared to determine effect of S1P1 heterozygosity on polyp number. Growth inhibition of RIE-1 cells was assessed using enforced expression of S1P1 receptor by adenoviral vector followed by treatment with S1P. Matched human normal and cancer colon tissue were obtained from surgical specimens.
Differential expression of S1P1 between the tissues was evaluated utilizing western blot analysis and immunohistochemistry.
Results: Bigenic S1P1+/−Apcmin mice revealed a 27% increase in polyp number when compared to control mice. Induced expression of S1P1 in RIE-1 cells caused growth inhibition with treatment of S1P.
Western blot analysis and immunohistochemistry revealed an increased expression of S1P1 in the human normal tissue as compared with tumor tissue.
Conclusions: Our results suggest that S1P1 receptor functions in the intestinal epithelium to inhibit tumorigenesis. Down-regulation of S1P1 in colorectal cancer may have functional consequences in the proliferation and or metastatic spread of cancer. Further evaluation of Sphingosine 1-phosphate receptor-1 is necessary to determine its potential for therapeutic intervention in colon cancer.

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Vitamin E succinate inhibits the in vitro growth of pancreatic cancer cells

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Background: Vitamin E Succinate (VES, α-tocopheryl succinate) is the most potent anti-tumor analog of Vitamin E that selectively induces apoptosis in cancer cells by modulating the expression of Bcl-2 family proteins. Despite its role being studied as a chemopreventive, chemotherapeutic and chemosensitizing agent in various cancers, there are scarce studies of VES in pancreatic cancer. Pancreatic cancer is the number four killer in the US and about 32,000 new cases are reported every year. The five year survival rate is only 5%. In this study, we investigated the effects of VES in three pancreatic cancer cell lines, ASPC-1, COLO-357 and PANC-1. We also assessed the synergistic growth inhibitory effect of VES along with two known cytotoxic drugs, Etoposide and Gemcitabine.
Methods: Cells were treated with varying concentrations (5 μM to 100 μM) of VES alone or in combination with Etoposide or Gemcitabine for different time periods. WST-1 cell proliferation reagent (Roche) was used to determine the cytotoxicity after the treatment. We studied the expression pattern of Bcl-2 family proteins in response to VES in ASPC-1 cells.
Results: VES inhibits the cell proliferation of all the three pancreatic cancer cell lines in a time and dose dependent manner. Our data also demonstrates that VES synergistically inhibits the cell growth in combination with 80 μM etoposide and 0.5 μg/ml Gemcitabine. In ASPC-1 cells, we observed a dose dependent decrease in the expression of Bcl-XL in response to VES.
Conclusion: This study demonstrates that (a) VES inhibits the *in vitro* growth of pancreatic cancer cell lines (b) Vitamin E succinate synergistically inhibits the growth of pancreatic cancer cells in combination with cytotoxic drugs Etoposide or Gemcitabine and (c) VES down-regulates the expression of antiapoptotic protein Bcl-XL in ASPC-1 cells.

Clinical methodology

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Preliminary results of a accelerated dose escalation phase I trial with a novel anthracycline derivative (RTA-744) in patients with primary brain tumors

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Background: RTA 744 is an anthracycline derivative that was shown in preclinical studies to cross the blood-brain barrier, not be a substrate for p-GP or MRP mediated efflux and improve survival in an orthotopic murine model of glioblastoma. A trial of RTA 744 was initiated at M. D. Anderson Cancer Center in patients with primary, high-grade gliomas.
Methods: RTA 744 is being administered as a 2-hour intravenous infusion on each of the first three days of a 21-day cycle. Dose escalation is proceeding according to an accelerated titration design, with single patient cohorts and 100% dose escalations until evidence of drug-related Grade 2 or greater toxicities are observed. Standard determinants of MTD are being employed. The MTD is being determined first in patients who do not take enzyme-inducing anti-convulsants. Pharmacokinetic samples are being taken at multiple time points on days 1–5 of Cycle 1. Tumor activity is being assessed according to the MacDonald criteria.
Results: As of May, 2006, RTA 744 has been administered to a total of 7 patients (pts) at dose levels of 1.2 (1 pt), 2.4 (3 pts), 4.8 (2 pts), and 9.6 mg/m^2/day (2 pts) (corresponding to 3.6, 7.2, 14.2, and 28.4 mg/m^2/cycle). No Grade $\geqslant$2 drug-related toxicities have been observed at doses of 4.8 mg/m^2 and below; results at 9.6 mg/m^2 have shown the first Grade 2 toxicities (platelets, lymphopenia and elevated SGPT). As a result the 9.6 mg/m^2/day cohort will be expanded and the percent of dose escalation for subsequent cohorts will be reduced. The pharmacokinetic profile indicates dose proportionality, with some accumulation by Day 3. Mean plasma half-life of RTA 744 thus far is approximately 34 hours. Three of the first four patients received at least four cycles, and one of these patients remains on study. Evidence of clinical activity was also seen in the first four patients, including 2 Minor Responses (2.4 mg/m^2) and 1 Stable Disease (1.2 mg/m^2). The most recent patient received a dose 4 times the level at which tumor regression was first documented. MRI results from the two most recently enrolled patients are pending.
Conclusions: RTA 744 is well tolerated up to doses of 9.6 mg/m^2/day, has predictable pharmacokinetics, and shows early evidence of activity. Full results of this trial should be available by the fall of 2006. Based on the activity seen to date, Phase 2 studies of this novel agent in primary and metastatic brain tumors appear warranted.

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Phase II trial of Sequenced Bevacizumab and Erlotinib with Bevacizumab and Chemotherapy for 1st Line Stage IIIB or IV Non-Small Cell Lung Cancer (NSCLC)

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Background: Recent evidence suggests that bevacizumab added to erlotinib increases activity in 2nd line metastatic NSCLC and the addition of bevacizumab to chemotherapy improves survival in 1st line metastatic NSCLC. Bevacizumab plus erlotinib (B+E) has never been tested in 1st line NSCLC. Furthermore, administration of 4 cycles of B+E prior to bevacizumab plus chemotherapy would allow selection of patients who could benefit from consolidation B+E.