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Clinicopathological and Prognostic Significance of p53 and TGF-Beta 1 in Patients With Gastric Cancer

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Background: The p53 protein is a molecule with numerous functions. However, mutation of p53 and accumulation of mutated p53 protein, which have no normal function, are common in human cancers. On the other hand TGF-beta1 may be implicated in the pathogenesis of the tumours, since it is expressed in neoplastic tissue.
The aim of this study is to investigate p53 value and it relationship with TGF-beta1 expression, clinicopathological parameters and survival in patients with gastric cancer.
Material and Methods: In 53 patients who had undergone gastrectomy for gastric cancer, the expression levels of p53 and TGF-beta1 in gastric cancer tissues were examined immunohistochemically (IHC). p53 gene alterations were examined by fluorescence microscope after in situ hybridization performing. Finaly the clinicopathological parameters such as gender, age, operation type, TNM stage and size of the tumour, histology and Lauren classification, and survival were analyzed retrospectively.
Results: We found p53 expression in all of gastric cancer specimens. Also, 47.2% of specimens were TGF-beta1 expression positive. Our data demonstrate a relationship between survival and p53 expression. The patients with p53 expression had worse prognosis after surgical therapy compared to those without. The median survival of p53-positive patients was 4.8 months whereas the median survival of p53-negative patients was 9.1 months ($p = 0.027$; log-rank test). Also, 88.9% from patients with p53-positive status is in T1-T2 stage vs. 37.8% in T3-T4 ($\chi 2 = 7.88$, $p = 0.005$). Finally we found that 56.1% from p53 expressive tumours have TGF-beta1 expression and 16.7% were non-expressive ($\chi 2 = 5.79$, $p = 0.016$). There was no statistically significant correlation between p53 expression and the other clinicopathological parameters.
Conclusions: In conclusion our results suggest that low expression of p53 and TGF-beta1could be useful as a marker of poor prognosis and had prognostic value for gastric cancer patients.

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Suppression of Stat3 Activity Sensitizes Gefitinib-resistant Non Small Cell Lung Cancer Cells

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Epidermal growth factor receptor (EGFR) is a proven therapeutic target to treat a small subset of non small cell lung cancer (NSCLC) harboring activating mutations within the EGFR gene. However, many NSCLC patients are not sensitive to EGFR inhibitors, suggesting that other factors are implicated in survival of NSCLC cells. Signal transducers and activators of transcription 3 (Stat3) function as transcription factor to mediate cell survival and differentiation and the dysregulation of Stat3 has been discovered in a number of cancers. In this study, we found that a small molecule, reactivation of p53 and induction of tumour cell apoptosis (RITA), showed anti-cancer activity against gefitinib-resistant H1650 cells through a p53-independent pathway. Stat3 suppression by RITA attracted our attention to investigate the role of Stat3 in sustaining survival of H1650 cells. Pharmacological and genetic approaches were employed to down-regulate Stat3 in H1650 cells. WP1066, a known Stat3 inhibitor, was shown to exhibit inhibitory effect on the growth of H1650 cells. Meanwhile, apoptosis activation by siRNA-mediated down-regulation of Stat3 in H1650 cells provides more direct evidence for the involvement of Stat3 in viability maintenance of H1650 cells. Moreover, as a novel identified Stat3 inhibitor, RITA increased doxorubicin sensitivity of H1650 cells in vitro and in vivo, suggesting that doxorubicin accompanied with Stat3 inhibitors may be considered as an alternative strategy to treat NSCLC patients who have inherent resistance to doxorubicin. Overall, our observations reveal that targeting Stat3 may be an effective treatment for certain NSCLC cells with oncogenic addition to Stat3.

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Antiproliferative and Apoptotic Effects of Black Currant Juice (Ribes Nigrum) on Lymphoblastic Leukemia Cells

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Background: Polyphenols are a group of naturally occurring compounds widely present in fruits and vegetables in human daily diets. These compounds have been reported to show anticancer and anti-mutagenic activities. Black currant juice (*Ribes nigrum*) is a rich source of polyphenols containing about 4.1 g/L. The aim of the present study was to determine whether black currant juice inhibits the proliferation of acute lymphoblastic leukemia cells (Jurkat cells) and, if so, to determine the underlying mechanism.
Material and Methods: Human acute lymphoblastic leukemia Jurkat cell line was used in the study. MTS assay, Cell cycle phase distribution and Apoptosis analysis were performed to study the effect of Black currant juice on proliferation, cell cycle and apoptosis in Jurkat cells respectively. The formation of ROS was determined by staining with dihydroethidine (DHE). Western blot experiments were performed to detect pJNK, p38 MAPK, pERK, pAkt, p73, Cyclin B1, Caspase 3 and UHRF1.
Results: Black currant juice inhibited the proliferation and induced cell cycle arrest in G2/M phase that led to a strong apoptotic effect. Cell cycle arrest and apoptotic effects were accompanied by an upregulation of p73 and caspase-3, and down-regulation of UHRF1. These findings indicate that black currant juice is a strong inducer of apoptosis in Jurkat cells. Mechanistic studies revealed that black currant juice significantly increased the formation of reactive oxygen species (ROS). The formation of ROS was accompanied with a strong upregulation of stress-related kinases (pJNK, p38 MAPK, pERK and pAkt) in a time-dependent manner. Intracellular inhibitors of ROS such as MnTMPyP, N-acetylcysteine and PEG-catalase inhibited the black currant juice-induced formation of ROS and upregulation of stress-related kinases.
Conclusion: The role of black currant juice-induced formation of ROS and upregulation of stress-related kinases in apoptosis still remains to be explored. These results indicate that black currant juice is a potent inducer of apoptosis in acute lymphoblastic leukemia cells and, hence, may be of potential in leukemia therapy.

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Antimycin A Sensitizes TRAIL-induced Apoptosis Through Down-regulation of C-FLIP and Bcl-2 Proteins

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Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) has been paid attention as a potential anti-cancer drug, because it induces apoptosis in a wide variety of cancer cells but not in most normal human cell types. Here, we showed that co-treatment with subtoxic doses of antimycin A (AMA), an inhibitor of electron transport and TRAIL induced apoptosis in human renal cancer cells, Caki cells, but not in normal tubular kidney cells. Treatment of Caki cells with AMA down-regulated c-FLIP and Bcl-2 proteins in dose- and time-dependent manners. AMA-induced decreases in c-FLIPL and c-FLIPs protein levels were involved in the increased protein instability, which was confirmed by the result that treatment with protein biosynthesis inhibitor, CHX, reduced c-FLIPL and c-FLIPs proteins level by AMA. We also found that AMA induced down-regulation of Bcl-2 at the transcriptional level. Pretreatment with N-acetyl-l-cysteine (NAC) slightly inhibited the expression levels of DR5 up-regulated by the treatment of AMA, suggesting that AMA appears to be partially dependent on the generation of ROS for up-regulation of DR5. Taken together, the present study demonstrates that AMA enhances TRAIL-induced apoptosis in human renal cancer cells by DR5 up-regulations, as well as cFLIP and Bcl-2 down-regulations.

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Gabexate Mesilate Induces the Apoptosis of HepG2cells

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Background: We happened to administer gabexate mesilate(GM)to patients with pancreatitis complicated by HCC. We found GM had a inhibiting effect on HCC. Therefore, an experimental study was performed to evaluate the mechanism of the inhibition.
Materials and Methods: HepG2 cells (HGC) were cultured. DNA fragments from cultured cells were extracted and electrophoresed. The cell were stained using TUNEL method. The immunohistochemical staining(IHCS)for P53, ss-DNA, bcl2 and caspase 3 (C3) in cultured for 24 hours were performed using polymer method with monoclonal antibody.

The cells were cultured with only GM and GM+C3 inhibitor. Then the number of cells was counted.
Results: The inhibition of HGC proliferation induced by GM was strongly found in time course and a dose dependent manners. In agar electrophoresis(EP), DNA extracts showed a negligible EP pattern. In the TUNEL the cell cultured with GM showed a decrease of total cell number(CN)and increase of positive CN. In IHC staining in P53, ss-DNA, bcl2 and C3 of GM treated cell culture (CC) for 24 hours, total CN decreased and positive cells increased. In the absence of GM, positive cells were scattered. The cell growth inhibition by GM was almost blocked by C3 inhibitor (Table 1).
Conclusions: The EP pattern often is seen in adhesive cells. The purpose of this study was to verify that the cell death is AP, not to investigate the activation rout of AP. To verify that GM causes the AP of cultured cells, it must be confirmed that there is a decrease in the cell count after the addition of GM on CC and that there is inhibition of a decrease in the cell count after the addition of GM+C3 inhibitor on CC. In this study AP was ascertained (Table 1). It was found that GM induced AP not necrosis of HGC. However,as HGC belongs to Type II cell, we speculate the following rout:P53 activation->BH3 activation $\rightarrow$ release of cytochrome c from mitochondria $\rightarrow$ Apf1 activation $\rightarrow$ C3 activation. TUNEL and IHCS showed the staining pattern supported AP. Perhaps, we think that when AP-promoting protein is more predominant than AP-inhibiting protein in the AP rout, AP may be induced. GM will be hoped as a good drug (perhaps to TAE & IV) that attacks to HCC by AP only with a little bit of side effects and without the effect to normal liver.

Table1. The effects of GM+C3 inhibitor on the proliferation of HGC

No.	Culture method	Total cell count
1	HGC for 24 hours culture	26.58×10^5
2	As 1 w/ GM 1,000 μM	18.46×10^5
3	As 2 + C3 inhibitor 80 μM	23.70×10^5 cells/ml

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P38 MAPK − a Potential Target for Metastatic Melanoma Therapy?

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Background: Metastatic melanoma (MM) is the most malignant of all skin cancers. Only a minority of patients respond to standard therapy, so the perspective for more efficient treatments lies in the development of new strategies for a selective inhibition of growth and elimination of MM cells. Overactivation of the ERK-MAPK signaling is frequently found in MM and is usually caused by mutations of Ras, B-Raf or PTEN. In contrast, the role of the p38 MAPK pathway in MM development and survival is poorly understood.
We have shown previously, that a small molecular inhibitor of p38 α/β MAPK SB202190 (SB) selectively induces autophagy in MM cell lines, but not in other cell types we tested. In our previous experiments, we have demonstrated that SB-induced autophagy promotes the survival of MM cells and combining SB with autophagy inhibitors can lead to a significant decrease of MM cell viability.
Material and Methods: Human melanoma cell lines A375, RVH-421, human osteosarcoma cells U2OS and human foreskin fibroblasts SCRC 1040 were cultured in sterile conditions. Light microscopy of living cells and viability assay with flowcytometry were performed after 24/48-hour of cultivation in the presence of selected inhibitors. Levels of antiapoptotic proteins were analyzed by western blotting.
Results: We investigated the effect of the BH3 mimetic gossypol on MM cell viability as a tool for maximizing the cytotoxic effect of SB and its combinations with autophagy inhibitors. In A375 cells this combination leads to the apoptotic death of nearly 90% of cells. Interestingly, the co-treatment with gossypol is sufficient for efficient induction of apoptosis in SB-treated A375 cells without the need for autophagy inhibitors. In contrast, in RVH-421 cells the addition of gossypol does not enhance the cytotoxicity caused by SB in combination with autophagy inhibitors. Interestingly, SB inhibits expression of Mcl-1 in RVH-421 but not in A375 cells and this might be the reason why further sensitization with gossypol is required for the triggering of cell death in A375 cells.
Conclusions: We showed that SB acts as a cellular stress inducer in MM cells to which they respond by inducing pro-survival autophagy. Our results indicate that increased expression of Mcl-1 protein may be a factor involved in the MM cells resistance to SB treatment and this resistance could be overcome with small molecule inhibitors such as gossypol.
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Re-expression of p16 Mediates Apoptosis in Cholangiocarcinoma With Low Rb Level

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Background: Cholangiocarcinoma (CCA) is a malignant bile duct epithelium which is a major liver cancer found in Northeast Thailand. The mortality rate of CCA is high while the survival rate is poor which results from advance stage of the patient at initial diagnosis. The tumour suppressor gene *p16* is a member of the INK4 family of cyclin dependent kinase inhibitor. It functions by direct binding to Cdk4/6 and preventing the phosphorylation of Retinoblastoma (Rb), which in turn blocks cell cycle transition from G1 to S phase. Our previous study showed that loss of p16 protein expression is the most frequent event in CCA (81.5%) and is significantly associated with poor survival. The present study aimed to address the role of re-expression of p16 in CCA cell lines harboring different levels of Rb using adenovirus system.
Materials and Methods: CCA cell lines KKU-100, M055, and M139 established from intrahepatic CCA samples were used in this study. These cell lines expressing no endogenous p16 were infected with p16 recombinant adenovirus vectors (*Ad-p16*) to mediate exogenous expression of p16. The Ad5CMV-Luc vector encoding luciferase was used as a control. Cell cycle and apoptosis were determined by Flow cytometry while beta-galactosidase associated senescence was performed using the X-gal staining method. Subcellular localization and protein levels of p16 and Rb were assayed using immunocytochemistry and Western blotting, respectively. *Rb* knockdown was performed using small interfering RNA (siRNA).
Results: Infection with *Ad-p16* resulted in significantly high level of p16 expression in all CCA cell lines. Exogenous p16 mediated senescence in M055 and M139 cells expressing high level of Rb while KKU-100 which expresses low level of Rb was undergone apoptosis. Apoptosis was observed in *Rb* knockdown M055 and M139 cells infected with *Ad-p16*.
Conclusions: Re-expression of p16 is capable of mediating apoptosis in CCA cell lines through low level of Rb expression and *Ad-p16* may be a promising candidate for cancer gene therapy in CCA.

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Protein-bound Polysaccharide From Phellinus Linteus Inhibits Tumour Growth, Invasion, and Angiogenesis Through Inhibition of Wnt/β-catenin Signaling in SW480 Human Colon Cancer Cells

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Background: Polysaccharides extracted from the *Phellinus linteus* (PL) mushroom are known to possess anti-tumour effects. However, the molecular mechanisms responsible for the anti-tumour properties of PL remain to be explored. In this study, the anti-cancer effects of PL were examined in SW480 colon cancer cells by evaluating cell proliferation, invasion and matrix metallo-proteinase (MMP) activity.
Background: Polysaccharides extracted from the *Phellinus linteus* (PL) mushroom are known to possess anti-tumour effects. However, the molecular mechanisms responsible for the anti-tumour properties of PL remain to be explored. In this study, the anti-cancer effects of PL were examined in SW480 colon cancer cells by evaluating cell proliferation, invasion and matrix metallo-proteinase (MMP) activity.
Material and metods: The anti-angiogenic effects of PL were examined by assessing human umbilical vein endothelial cell (HUVEC) proliferation and capillary tube formation. The *in vivo* effect of PL was evaluated in an athymic nude mouse SW480 tumour xenograft model.
Results: PL (125–1000 μg/ml) significantly inhibited cell proliferation and decreased β-catenin expression in SW480 cells. Expression of *cyclin D1*, one of the downstream-regulated genes of β-catenin, and T-cell factor/lymphocyte enhancer binding factor (TCF/LEF) transcription activity were also significantly reduced by PL treatment. PL inhibited *in vitro* invasion and motility as well as the activity of MMP-9. In addition, PL treatment inhibited HUVEC proliferation and capillary tube formation. Tumour growth of SW480 cells implanted into nude mice was significantly decreased as a consequence of PL treatment, and tumour tissues from treated animals showed an increase in the apoptotic index and a decrease in β-catenin expression. Moreover, the proliferation index and microvessel density were significantly decreased.