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Influence of wortmannin on non-homologous DNA end joining in human normal and cancer cells

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DNA double-strand breaks (DSBs) are the most genotoxic lesions. If they are not repaired they may lead to cell death, if mis-repaired they may result in mutations or cancer transformation. Human cells repair DSBs mainly via the non-homologous end joining (NHEJ) pathway. DNA-PKcs kinase activity plays an important role in this pathway. We have employed an in vitro assay to study the role of DNA-PKcs in NHEJ reactions. In this method, fluorescent dye allowed for direct visualization of rejoined linearized plasmids by human cell extracts. We used the DNA-PKcs kinase inhibitor wortmannin to measure the rejoining sensitivity of cell extracts to the drug. Our findings demonstrate that rejoining by human normal and cancer cells is relatively insensitive to wortmannin under the conditions of our assay. Moreover, DNA-PKcs immunodepletion resulted in only a modest reduction of end joining. In conclusion, our data suggest that under specific defined in vitro NHEJ reaction conditions, the presence of DNA-PKcs is not stringently required for production of end joined products.

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Determination of some biochemical effects associated with boron neutron capture therapy in experimental hepatomas

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Background: Boron neutron capture therapy (BNCT) is based on the selective delivery of boron-10 (^{10}B) to tumor cells. Following irradiation with low-energy neutrons, nuclear capture and fission reactions occur that produce He^{2+} and Li^{3+} particles. The effectiveness of BNCT is dependent upon the amount of ^{10}B deliver per cell. Approximately 10^9 ^{10}B atoms per tumor cell are necessary to produce four to five particles per cell, but studies of radiation-induced apoptosis suggest that BNCT also may be cytotoxic via other mechanisms so that the required number of ^{10}B atoms actually may be less. The aim of our paper is to find new biochemical mechanisms such us the oxidative destruction involved in tumoral cell cytotoxicities.
Materials and Methods: RS1 hepatoma-bearing rats were given single i.p. injection of 30 mg ml^{-1} of a BPA: fructose 1.0:1.1 molar solution. Mice were euthanized 1, 3, or 6 h after the injection. Tumor, blood, and liver were assayed for boron biodistribution and oxidative stress parameters Than the tissues was irradiated 2700 seconds with a 1.472×10^5 n/cm^2 epitermal fluency beam.
Results: Our results show preferential capture of BPA at tumoral level with a maximum value at 3 hours after the administration, the number of 10B atoms calculated in one gram of tumoral tissue is ranging between 10^{12} and 10^{17} atoms, the highest value of BPA internalization in tumoral cells is in the range of 20–40 μg. The lipid peroxides level measured in blood after the BPA administration is increasing two times at the hepatoma bearing rats than in normal control, also the caeruloplasmin Cu-oxidase activity growth from 168 I.U. to 330 I.U., the albuminic thiol-groups are decreasing from 267 μmol/l at 107 μmol/l
Conclusions The BPA administration possibly induce methabolic pathways wich involves the oxigen consumption, and after the irradiations the cytotoxicities is done by oxigen free radical production The biochemical parameters of oxidative stress can be used in monitoring the evolution of hepatoma, the modifications after BPA administration and possible the irradiation effects

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Inhibition of thymidine phosphorylase decreases tumour aggressiveness but reduces chemosensitivity in liver fluke related cholangiocarcinoma

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Background: Liver fluke related cholangiocarcinoma (CCA), the most common malignancy in the Northeast Thailand, is an important public health problem because the incidence and the fatality rate are high. Thymidine phosphorylase (TP) gene has been shown to be amplified (53.8%) in CCA of our previous report suggesting that TP may play an important role in carcinogenesis or pathogenesis of CCA.
Materials and Methods: We evaluated the role of TP by RNA interference (RNAi) using small interfering RNA (siRNA) directed against the human TP mRNAs in KKU-M139 CCA cell line, which has naturally high level of endogenous TP. TP-siRNA knockdown cells were tested for functions in vitro.
Results: siRNA targeting of TP dramatically impaired the expression up to $87.1 \pm 0.49\%$ of mRNA and $72.5 \pm 3.2\%$ of TP protein compared with those of control. We have demonstrated that TP depletion by siRNA reduced TP-induced proliferation and migration of KKU-M139, and suppressed TP activity on inducing migration and tube formation of human umbilical vein endothelial cells (HUVECs). siRNAs also interfered the ability of TP on resistance to hypoxia-induced apoptosis but not UV-induced apoptosis of KKU-M139. On the other hand, suppression of TP reduced the response of KKU-M139 to 5-fluorouracil (5-FU) chemotherapy. However, combination of siRNA knockdown and UV exposure significantly decreased the concentration of 5-FU required to inhibit cell proliferation compared with that of siRNA alone.
Conclusions: We suggest that it is useful to examine expression level of TP in tumour tissues for selecting patients who are likely to response to 5-FU. Since TP increases tumor aggressiveness and there are several chemotherapeutic drugs of choice, inhibiting TP activity by such tools (siRNA) may be a good benefit for improving the poor prognosis of cancer patients who show high expressions of TP.

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Quantitative prediction of cancer drug efficacy by AKT and ERK1/2 down regulation in pancreatic cancer

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Cancer drugs are increasingly designed to target specific cell-signaling pathways. However, the pathways governing apoptosis in mammalian cancer cells are complex, and the pro- and antiapoptotic permutations are related to cell viability and resistance to cancer drugs according to species, cell types and also since they can be activated at different points. Therefore, it is hard to predict the best treatment for a particular tumor. Here, we examined the key enzymes quantitatively working as activation or inhibition by various cancer drugs among individual patients. As cancer cells, we used highly malignant human-derived pancreatic cancer line, MIA PaCa-2, PANC-1 and Suit-2 because pancreatic cancer cure is unusual with cancer recurrence as metastatic disease in many cases after removal surgery of the primary tumor. For cancer drugs, a recombinant humanized anti-HER2 antibody (Herceptin) as a practically used example and plant-derived phenolic compounds of quercetin, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one and trans-resveratrol, 5-[(1E)-2-(4-hydroxyphenyl)-ethenyl]-1,3-benzenediol to prevent cancers as daily digested food examples, respectively. First, we examined the receptor expression on cell membrane and the different level of expression was observed. Secondary, we cultured cells with various concentrations of cancer drugs for 48 h and observed the concentration dependent drug efficacy together with the different magnitude among cell lines as cell viability which related to caspase-3 activity implying apoptosis. Thirdly, we assayed enzymes after 24 h of cell culture with a given cancer drug and found the positive linear relationships between relative activity of AKT protein phosphorylation [pS473] and cell viability as $r = 0.941$, and between relative activity of both dual phosphorylation ERK2 [pTpY185/187] and ERK1 [pTpY202/204] and cell viability as $r = 0.959$ by ELISA using all cell lines and cancer drugs, respectively. These results suggest that the down regulation of both MAPK and AKT/PI-3K pathways quantitatively relates to cancer drug efficacy

regardless their molecular mechanisms. Furthermore, we found the positive linear relationship between relative activity of AKT and ERK1/2 combined and cell viability as r = 0.948 in the same manner. The results obtained suggest quantitative cross-talk between the main two pathways regardless molecular mechanisms, which may aid cancer drug selection to a patient.

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Human papillomavirus (HPV) infection, p53 overexpression and histopathologic factors in colorectal cancer

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Background: There is evidence of a possible etiological role of human papillomaviruses (HPVs) in the development of colorectal cancer. Loss of p53 tumor suppressor gene function has been found in many malignancies and it can occur in a variety of ways, including gene mutation and interaction with the E6 protein of oncogenic human papillomaviruses (HPVs). The aim of this study was to verify the prevalence of HPV infection and p53 overexprssion in colorectal cancer tissue samples and its association with histopathologic factor.
Materials and Methods: Sixty tissue sections from CRC patients were investigated immunocytochemically for aberrant expression of p53 using the streptavidin-biotin-peroxidase method with monoclonal antibodies. HPVstatus was also analyzed using type-specific primers for HPV16/18 by polymerase chain reaction (PCR).
Results: Overall, 21of 60 patients (35%) presented HPV DNA; HPV 18 was detected in 19of 60 samples (31.7%) and HPV16 in 11 of 60 (18.3%). An abnormal expression of tumor-suppressor protein p53 were observed in 29 0f 60 (48.3%) samples. P53 overexpression was observed in 15/21 (71.4%) of HPV positive and 14/39 (35.8%) of HPV negative patients (P = 0.009). Same significant difference were found between HPV18 and p53 (P = 0.007) but not in HPV16 (P = 0.261). HPV DNA presentation was not significantly associated with histopathologic factor including tumor stage (P = 0.428), grade (P = 0.668), PNI (P = 0.265) and LVI (P = 0.275).
Conclusion: Our results suggest that p53 inactivation caused by HPV infection may play a role in the pathogenesis of colorectal cancer but there is not any association between HPV infection with histopathologic factor.

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Effects of cisplatin exposure on the expression of Bcl-2-family proteins: differences between cisplatin-sensitive and -resistant malignant pleural mesothelioma cells

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Background: Resistance toward apoptosis is one of the hallmarks of cancer, and cancer therapy failure is often attributed to apoptosis resistance. Malignant mesotheliomas (MM) are aggressive tumors that frequently acquire drug resistance during treatment. The chemotherapy regimens available often include the chemotherapeutic drug cis-diamminedichloroplatinum(II) (cisplatin, CDDP). In MM, there is evidence that the apoptosis-resistant phenotype is a consequence of suppressed mitochondrial membrane permeabilisation (MMP). The Bcl-2 family of proteins, which includes both pro-apoptotic proteins (e.g. Bim, Puma, Bid, Bad, Bmf) and pro-survival proteins (e.g. Bcl-2, Bcl-XL), is essential for the regulation of the MMP. We compared a malignant pleural mesothelioma cell line (P31wt) with its CDDP-resistant sub-line (P31res) regarding CDDP effects on the expression of Bcl-2-family proteins.
Materials and Methods: After 0.5, 2 or 6 h CDDP exposure, protein expression in cell lysates was determined with Western blotting. Equitoxic concentrations, 10 mg/L (P31wt) and 40 mg/L (P31res), of CDDP were used: 72 h after a 6-h exposure to CDDP, 50% of the cells had died of apoptosis, as determined by TUNEL staining.
Results: Under control conditions, some proteins were differently expressed: (1) the P31res cells did not express the most potent isoform of Bim; (2) P31res cells had a reduced expression of Puma; and (3) the P31res cells had a higher expression of P-Bcl-2 and P-Bad. In P31wt cells, which have a primary resistance toward CDDP compared to many other cancer cell lines, CDDP exposure (1) increased the expression of Bim and Puma, (2) increased the expression of Bad, and (3) decreased the expression of Bcl-2. In P31res cells, which have an acquired resistance toward CDDP, CDDP exposure (1) increased the expression of Puma, (2) decreased the expression of P-Bad, and (3) decreased the expression of pro-survival Bcl-2 and Bcl-XL.
Conclusions: Compared to P31wt, the P31res cells had lower expression of potent pro-apoptotic proteins and higher expression of P-Bad and P-Bcl-2. Cisplatin exposure reduced the expression of pro-survival proteins in both cell lines, but the effect on pro-apoptotic proteins differed: in P31wt most of the pro-apoptotic proteins increased, in P31res cells only Puma expression increased. These results suggest that the regulation of pro-apoptotic proteins can have an important role in CDDP resistance.

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Clinical requirements of "In Silico Oncology" as part of the integrated project ACGT (Advancing Clinico-Genomic Trials on Cancer)

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Background: New methods and technologies in molecular biology will result in an exponential increase of information that can be handled by the advances of high-computing and informatics. It is of paramount importance to gather this information with clinical data to gain new knowledge for developing better and more individualized treatments for cancer patients. This approach results in clinico-genomic trials, as ACGT (Advancing Clinico-Genomic Trials on Cancer) is running.
Materials and Methods: Substantial efforts have been made in mathematically simulating tumour growth and response to treatment resulting in a discipline called In Silico Oncology. Such in silico experiments might help clinicians to find the best way of treating an individual patient by simulating different treatments in the computer before starting the treatment in reality.
Results: From a clinical point of view two preconditions are of utmost importance, before a physician can rely on predictions of in silico simulations:
1. every in silico experiment has to be part of a clinico-genomic trial
2. every prediction of an in silico experiment has to be compared with the reality.

In the process of developing in silico experiments it is necessary to define the necessary and available data in a first step, including data from the tumor (molecular biology, pathology, imaging), from the patient (clinical data) and from possible treatments (pharmacokinetics of drugs, the treatment schema). Because the amount of data is restricted by the availability of tumour material, imaging data and clinical data, In Silico Oncology has to be part of clinico-genomic trials based on GCP criteria.
The simulation prediction of each in silico experiment has always to be compared with the reality. Only if there are no or minimal deviations between the prediction and the reality the in silico experiment is allowed to be used in a clinical setting. Before an in silico experiment can be accepted as a routine method for treatment stratification, a prospective and randomised trial has to show that patients treated according to the result of the in silico simulation experiment do better, than those treated regardless of the result.
Conclusions: In ACGT in silico models of breast cancer and nephroblastoma are tested regarding tumour growth and response to treatment.
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Analysis of coding and non-coding regions of thymidylate synthase gene in colorectal cancer patients and its possible relationship with 5-fluorouracil drug response

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Background: Thymidylate synthase (TS) catalyzes methylation of dUMP to dTMP and is the target of 5-fluorouracil (5-Fu). TS gene has regulatory tandemly repeated sequences in its 5′ and 3′ untraslated regions (5′-3′ UTR). TS levels vary considerably among tumors and the response to 5-FU is influenced by the intratumoral activity of the enzyme, with high levels generally being associated with a poor response. A recently detected 6 bp deletion polymorphism in the 3′UTR of the TS gene might also