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Effect of selected chalcones on cancer cells

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Chalcones are precursors of flavonoids in their biosynthetic pathway. These conjugated molecules possess great conformational flexibility. Variety of biological activities have been demonstrated for these compounds such as anti-inflammatory, analgesic, antiviral (including anti-HIV), antibacterial, gastroprotective, antioxidant as well as cytotoxic properties depending on the character and position of substituents on their aromatic ring. However, there is only a limited amount of literature concerned with antiproliferative effects of chalcones.

In the present work, we tested two newly synthesized dimethoxybenzylidene chalcones Q-510 and Q-705 for their cytotoxic and antiproliferative effects on HeLa cells at concentrations of 1, 10 and 100 mmol.l-1. Effects of these compounds were tested by employing MTT cytotoxicity assay, flow cytometric analysis of the cell cycle by using PI staining and apoptosis by flow cytometric analysis (annexin V/PI staining) or DNA fragmentation.

Our data indicate higher cytotoxic effect of Q-510 in comparison with Q-705. Incubation of HeLa cells with Q-510 and Q-705 at 1 mmol.l-1 for 72h caused 45% and 20% reduction in cell survival, respectively, as determined by MTT assay. Cell proliferation was inhibited in a dose-dependent manner. Cell cycle analysis revealed initial G2/M arrest in both Q-510- and Q-705-treated HeLa cells followed by an increase in the sub-G0/G1 fraction. The presence of apoptosis was also confirmed by annexin V/PI staining and DNA fragmentation.

The present study demonstrates antiproliferative and cytotoxic properties of two newly synthesized dimethoxybenzylidene chalcones: Q-510 and Q-705. Q-510 turned out to be a more effective agent than Q-705. Further studies are necessary to elucidate its mechanism of action, nevertheless, this compound might have a potential to enter pre-clinical trials as a new anticancer drug.

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High throughput experimental verification of predicted tissue and tumor specific splice isoforms

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Alternative splicing of transcripts may lead to different mRNA species and therefore to potentially different proteins. Any failure or error in the splicing control mechanism can be involved in a number of pathological processes, such as cancer. Therefore, splice isoforms that are disease specific could serve as excellent diagnostic markers, which are easily identifiable by PCR. Computational prediction of alternative splice variants has been highly facilitating the identification of novel splice isoforms.

Our prediction strategy is based on the genomic mapping (SpliceNest) of EST consensus sequences and library annotation provided in the GeneNest database. This revealed 427 genes with at least one tissue specific transcript as well as 1120 genes showing tumor specific isoforms. Out of these genes, a subset of predicted isoforms were experimentally verified by an RT-PCR screening approach. We have set up an experimental strategy that allows us to screen expression of genes in up to 112 different human tissues of multiple developmental stages and cell lines.

Within this project, the electrophoretic separation of RT-PCR products turned out to be the bottleneck impeding the switch from a medium to a high throughput strategy. To circumvent the limitations of DNA slab gel analysis, a lab prototype of an automated on-chip electrophoresis system that allows high throughput analysis of DNA fragments was implemented in the workflow. In our experimental set-up, we analyzed RT-PCR samples on 4×96 well plates within a defined sequence of consecutive one-on-one measurements. The high throughput experimental verification of computationally predicted tissue specific isoforms revealed a high success rate in confirming their expression in the respective tissue. However, low expression levels of the respective transcript and the limited sensitivity of the experimental method can explain failed detection of the restricted expression pattern. The combination of computational prediction of alternative splicing events with high throughput experimental verification facilitates the efficient detection of tissue and tumor specific transcripts.

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Transcriptional profiling in human epidermal squamous cell carcinoma outlines tumor-specific gene expression signatures

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Background: The transcriptional spectrum of cell types within the epidermal squamous epithelium poses an interesting challenge in describing gene expression profiles for squamous cell carcinoma (SCC). To define molecular links within the cascade of genetic elements and environmental interactions that coordinate the initiation and progression of the neoplastic process we determined gene expression directly in the skin of patients with SCC.

Materials and Methods: We report profiles in 5 SCC patients using Affymetrix® Human Genome U95A (12,627 sequences) arrays that distinguish SCC affected versus unaffected skin, representing the most complete microarray study to date in human tumor tissue.

Results: Examination of specific genes within these expression signatures provides evidence for loss of structural integrity, increased angiogenic potential, modification of intracellular signal transduction, cell cycle disturbance via p53 disruption, and anti-apoptotic related mechanisms of disease. Several novel genes involving the p53 pathway, anti-apoptotic pathways, signal transduction, structural loss, and DNA replication, including BCL2A1, MUC4, SHP2, and FGF9 were found to be up-regulated in SCC tissue.

Conclusion: The vast majority of differentially expressed genes were found to be involved in functional pathways relating to cellular transformation. These differentially expressed genes may encompass diagnostic and/or prognostic determinants of SCC. Overall, this study supports the prevailing hypothesis that SCC pathology is the result of a collection of disrupted cellular processes cumulatively resulting in a disease state.

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Protective effect of flavonoids from Garcinia kola seeds on D-galactosamine induced toxicity in mice

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Background: *Garcinia kola* seed is commonly consumed in West Africa and used as remedies for the treatment of cough, laryngitis and liver diseases. In an attempt to reconcile claims of the beneficial effects of this seed, the protective effect of flavonoids viz; kolaviron (KV), *Garcinia biflavanone* (GB) 1, GB 2 and kolaflavanone (KF) from the seed was tested in D-galactosamine ($GalNH_2$)-intoxicated mice.

Materials and Methods: Thirty-five male TO strain Mice was divided into seven groups of five animals each. Groups 1–4 were administered KV, GB1 GB2 and KF at a dose of 100 mg/kg body weight/day for seven consecutive days and then challenge with a dose of D-$GalNH_2$ (800 mg/ kg). Groups 5 and 6 serve as negative control (received $GalNH_2$ alone) and corn oil (drug vehicle), respectively. The animals were sacrificed 24 hours after $GalNH_2$ administration.

Results: Pretreatment with KV, GB1, GB2, and KF before challenge with a single dose of $GalNH_2$ (800 $mgKg^{-1}$) significantly ($P < 0.05$) decrease serum alanine and aspartate aminotransferases activities by over 60% and 35%, respectively when compared with $GalNH_2$-only group. In addition, pretreatment with KV, GB1, GB2 and KF significantly ($P < 0.05$) decrease the $GalNH_2$ – mediated increase in the activity of microsomal γ-glutamyl transferase by 42%, 64%, 28% and 22% respectively, and prevented the accumulation of hepatic malondialdehyde and the depletion of reduced glutathione as obtained in the liver of $GalNH_2$-only intoxicated mice. KV, GB1, GB2, and KF caused insignificant ($p > 0.05$) effect on the levels of cytochrome P450 2EI and bcl_2 protein assayed by western blotting technique. Whereas the pretreatment with these flavonoids caused an induction of glutathione-S-transferase activity by over 34%. $GalNH_2$-induced hepatotoxicity was essentially prevented as indicated by a liver histopathologic study of mice treated with GB1 and GB2.

Conclusion: Our results suggest that KV, GB1 and GB2 (flavonoids of *Garcinia kola*) protect against $GalNH_2$-induced hepatotoxicity. This protection may be due to their ability to induce the expression of phase II drug metabolizing enzymes.

Keywords: *Garcinia kola*, Galactosamine, Cytochrome P450, Toxicity.