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Assessment of the dipeptidyl peptidase-IV inhibitory activity of protein hydrolysates *in silico* and *in vitro*

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Backgrounds: Recently, dipeptidyl peptidase-IV (DPP-IV) inhibitors that protect active GLP-1 from being cleaved by DPP-IV have been used as the new management of type 2 diabetes. Protein hydrolysates were also found to inhibit DPP-IV, and mostly comprised proline or alanine as the penultimate N-terminal residue (Xaa-Pro and Xaa-Ala). The aim of this study was to evaluate the DPP-IV inhibitory activity of the hydrolysates *in vitro* and predict the amino acid sequence of the active peptide *in silico*. **Materials and Methods:** i) *In silico* analysis: protein sequences were searched from ExPASy database. Enzyme actions were predicted using BIOPEP database that would theoretically produce the peptides from protein. Then the equation following equation was used to determine the occurrence frequency of Xaa-Pro and Xaa-Ala: Frequency = number of peptides as Xaa-Pro and Xaa-Ala /number of total peptides. ii) *In vitro* study: sodium caseinate (casH)、isolate soybean protein (soyH) and isolate wheat protein (whtH) hydrolysates were prepared using Bromelain (Br) (E/S=5%; 45°C60min); Thermolysin (Th) (E/S=3%; 70°C; 20min) and simulated gastrointestinal digestion (Dig) (pepsin、trypsin、pancreatin; E/S=5%; 37°C). The hydrolysates with high DPP-IV inhibitory activities were fractionated by ultrafiltration of molecular weight cutoffs of 2.5 kDa and 1 kDa. **Results:** The higher scores of frequency were found in cash than the other two hydrolysates, therefore we suggested that casein is the good source to make DPP-IV inhibitor peptides. The samples with the higher inhibitory activity (5mg/mL) were Br-casH (72%)、Th-soyH (69%) and Dig-casH (64%), and three samples were further fractionated by ultrafiltration. We found that all the fractions < 1 kDa of the three hydrolysates were shown to possess the highest DPP-IV inhibitory activity, and that of Br-casH had the greatest inhibitory rate of 77.5%. According to the results, we suggested Br-casH within <1 kDa had the potential to be the DPP-IV inhibitor due to the high amount of Xaa-Pro and Xaa-Ala peptides. **Conclusion:** In this study, we confirms Br-casH within <1 kDa had the highest inhibitory activity and the potential to be the source of antidiabetic peptides.

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Hedgehog-Interacting Protein (HHIP) Is A Key Repressor of Hedgehog Signaling That Drives Cell Survival, Invasion, and Drug-Resistance in Lung Adenocarcinoma

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Backgrounds: Stemness genes and pathways play important roles in embryonic development and adult tissue regeneration. Recent years, the aberrant activations of stemness signaling such as Hedgehog (HH), hypoxia-inducible factor (HIF), and Wnt pathways, and the stemness factors like Oct-4 and Sox-2 have been reported in lung cancers. However, most researches to date focused on the impact of positive regulators of stemness pathways in oncogenesis, but less on the importance of negative regulators. Hedgehog interaction protein (HHIP) is a membrane protein that binds to HH ligands with an affinity comparable to Ptch-1 (the native HH ligand receptor), and HHIP overexpression attenuates HH signaling by capturing HH ligands. HHIP has been found to be down-regulated in several types of cancer cell lines or tumor samples through promoter hyper-methylation. In NSCLC, however, its role and importance has not been identified. **Materials and Methods:** NSCLC cell lines including H1975, H358, and HCC827 were used in this study. We compared the invasiveness, proliferation rate, and colony forming ability between HHIP overexpressing cells and control cells under normal culture condition and starvation state. RT-PCR and western blotting were performed to find the signals activated during starvation state. **Results:** Here, we show that HHIP was significantly repressed in lung cancer cell lines and human lung tumor samples through epigenetic silencing. Overexpression of HHIP in lung cancer cells blocked the auto-loop induction of endogenous HH pathway, and inhibited the invasiveness of cancer cells. We also found that in starvation state, proliferation rate of HHIP overexpressing cells was reduced, as compared to control. Furthermore, starvation induces autonomous HH pathway activation which then mediated the expressions of HGF, and overexpression of HHIP repressed such inductions. This HGF induction also correlated to decreased sensitivity to Genfinitib treatment. **Conclusion:** In summary, our results indicate that the negative regulator of stemness pathway can be silenced in lung cancer cells and thus potentiate the cells to activate stemness pathway to acquire the abilities of survival, metastasis, and drug-resistance in adverse environments.

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Hsa-miR-876-5p is down-regulated in the Enterovirus 71 persistent infected neuroblastoma cells

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Backgrounds: Enterovirus 71(EV71) is a single-strand and positive sense RNA virus. EV71 belongs to the genus of enteroviruses in the Picornaviridae family. The RNA genome is about 7,500 nucleotides. EV71 is reported one of the major causative agents for hand- foot-and-mouth disease (HFMD) and emerge as a major cause of neurological disease involving death among young children worldwide. MicroRNA is a small noncoding endogenous RNA molecule, which function is posttranscriptional regulation of gene expression. Human miRNAs have recently been found to have important roles in viral replication. **Materials and Methods:** EV71 persistent infected neuroblastoma SF268 cells were established in this study. The global profiling of miRNAs were identified and compared from the EV71 acute and persistent infected human neuroblastoma SF268 cells by next generation sequence (NGS). In addition, oligonucleotide microarray was employed for the identification of differentiated expression of the host genes from EV71 persistent infected SF268 cells. **Results:** Many microRNAs were identified either up-regulated or down-regulated in the EV71 persistent infected SF268 cells. We chose miR876-5p for further characterized of the functional role(s) in EV71 infection cycle. miR-876-5p was significant down-regulation in the persistent infected SF268 cells when compared with the mock-infected cells. By contrast, there was no difference between the acute infection and mock-infected SF268 cells, indicating that the reduction of miR-876-5p plays role in the establishment of EV71 persistent infection. CREB5, the cAMP response-element-binding protein, was significantly increased in EV71 persistent infected SF268 cells, indicating this gene may be correlated with miR-876-5p. **Conclusion:** We dedicate to investigate the potential function role and involved pathways driven by miR-876-5p. Finally, the functional role of miR-876-5p involved in the EV71 persistent will be discussed in this study.

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Quinacrine Induces Apoptosis of Human Leukemia K562 Cells via Bcl-xL Down-regulation

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Backgrounds: Quinacrine, also known as mepacrine, is a drug applied on anti-protozoal, anti-rheumatic therapy, and functioned as an intrapleural sclerosing agent as well. Recently, the anticancer activity of quinacrine has been report. In this study, we aimed to investigate the mechanism responsible for quinacrine-induced cell death in human leukemia K562 cells. **Materials and Methods:** After treatment with 10 μM quinacrine for suitable time intervals, the cell death signaling pathway was detected using RT-PCR, flow cytometry and ELISA assay. Moreover, regulation of Bcl-xL expression in quinacrine-treated cells was analyzed by western-blotting assay, RT-PCR and promoter luciferase assay. **Result:** Quinacrine induced K562 apoptosis in a concentration-dependent manner. Quinacrine-induced ROS generation led to p38 MAPK phosphorylation, ERK inactivation and Bcl-xL down-regulation. Reduction in Bcl-xL mRNA level and promoter luciferase activity elucidated Bcl-xL protein decrease and loss of $\Delta\Psi m$ in quinacrine-treated cells. Suppression of phospho-p38 MAPK or transfection of constitutively active MEK1 abolished quinacrine-induced Bcl-xL down-regulation and $\Delta\Psi m$ loss. N-acetylcystetine (ROS scavenger) pretreatment abrogated the effect of quinacrine on the $\Delta\Psi m$ loss, and the levels of phospho-p38 MAPK and phospho-ERK. **Conclusion:** This study suggests that quinacrine induces apoptosis of human leukemia K562 cells via Bcl-xL down-regulation.