PHOSPHORYLATION AND REGULATION OF HUMAN CHOLINE KINASE BETA BY PROTEIN KINASE A

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Introduction: Choline kinase (CK) is the first enzyme involved in CDP-choline pathway for the biosynthesis of phosphatidylcholine, the major component of membrane phospholipid. CK exists as three isozymes, which are CKα1, CKα2 and CKβ. The regulation of these enzymes is physiologically important. Metabolic alterations of CKα are associated with tumorigenesis, while mutation or deletion of ckβ gene leads to the development of muscular dystrophy. In anticancer research, inhibition of CK activity has been explored as a potential therapeutic strategy.

Objectives: Post-translational modification is one of the mechanisms to regulate the function of CK. Growing evidences support that yeast and human CKα are regulated by phosphorylation but the phosphorylation of CKβ has never been reported. Hence, the general objective of this work was to study the phosphorylation and regulation of CKβ.

Methods: In this study, protein kinase A (PKA) was identified as the protein kinase responsible for the phosphorylation of CKβ by in-gel kinase assay. PKA phosphorylation was confirmed with specific PKA inhibitor and Western blotting. In vitro assay with commercial PKA further supported CKβ as the substrate for PKA phosphorylation.

Results: The phosphorylation occurred at serine 39 and 40 residues in the N-terminal region of CKβ. Phosphorylation of CKβ was observed in human embryonic kidney cells (HEK293) and liver hepatocellular carcinoma cells (HepG2). Forskolin and 3-isobutyl-1-methylxanthine treatment increased the phosphorylation level of CKβ, while the phosphorylation was inhibited by PKA inhibitor (H-89). The phosphorylation level of CKβ was also increased by epidermal growth factor. The effects of PKA phosphorylation on the biochemical properties of CKβ were subsequently examined. PKA phosphorylation increased the catalytic activities of CKβ with choline, ethanolamine and ATP as substrates. The Vmax values for choline, ethanolamine and ATP were increased by 47.1%, 81.8% and 50.8%, respectively. PKA phosphorylation improved the affinity of CKβ for choline and ATP, but decreased the affinity of CKβ for ethanolamine. Consequently, the catalytic efficiencies of CKβ for choline and ATP were increased by 121.0% and 97.5%, respectively. The same effects of PKA phosphorylation on the biochemical properties of CKβ were mimicked by double mutation of the phosphorylated serines to aspartates. PKA phosphorylation also dramatically increased the sensitivity of CKβ to hemicholinium-3 (HC-3), a potent inhibitor of CK. The IC50 value for phosphorylated CKβ (50 μM) was 29 times lower than the unphosphorylated enzyme (1.45 mM). In addition, PKA phosphorylation also decreased the stability of CKβ protein against urea denaturation. On the contrary, phosphorylation did not affect the optimum pH, subcellular location and oligomeric state of CKβ.

Conclusion: This study reports the phosphorylation and regulation of CKβ by PKA for the first time. The knowledge provides new insight into the intracellular regulation of CKβ catalytic properties by phosphorylation that might be an important mechanism to modulate lipid metabolism and cell growth.

Supervisor: Associate Professor Dr Few Ling Ling
Co-supervisor: Dr Khoo Boon Yin

EVALUATION OF DNA METHYLATION EFFECT ON CpG-ISLAND CONTAINING PROMOTER OF CHOLINE KINASE BETA

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Introduction: Choline kinase (CK) is the first enzyme in CDP-choline pathway, catalyzes phosphorylation of choline to phosphocholine (PC) in the presence of ATP and Mg2+ during de novo biosynthesis of phosphatidylcholine, the major eukaryotic membrane phospholipid. Human CK is encoded by two separate genes, ckα and ckβ which encode three different polypeptides, CKα1, CKα2, and CKβ. Apart from the involvement in PC biosynthesis, loss of ckβ gene was also associated with autosomal recessive congenital muscular dystrophy with mitochondrial structural abnormalities in human and marine.

Objectives: Previous studies showed ckβ promoter as a TATA-less, GC-rich promoter which led to the assumption that epigenetics regulation at the promoter through DNA methylation might regulate the expression of ckβ gene. In this study, DNA methylation status on the second CpG island of
ckβ promoter was analysed to verify the effect of methylation on ckβ promoter.

**Methods:** Semi-quantitative measurement of restriction-refractory fragment template amplification with endpoint PCR amplification and quantitative real-time PCR amplification methods were performed to analyze the DNA methylation status on the second CpG island of ckβ promoter in HepG2 cell line that was subjected to a DNA demethylating agent (5-Azacytidine, 5-Aza) and a hypermethylating agent (budesonide).

**Results:** Restriction enzyme analysis showed that isoschizomer pair methylation sensitive/dependent restriction enzyme (MSRE/MDRE) recognition sites were found at -769 and -899 whereas MSRE HhaI recognition site was found at -714 on the second CpG island of ckβ promoter. The baseline DNA methylation analysis at -769 and -899 revealed a presence of higher amount of methylcytosine (mC) than unmodified cytosine (C). Both findings shows all the three recognition sites as highly methylated, suggesting the second CpG island of ckβ promoter was highly methylated at its normal condition. To study the effect of epigenetic modification on DNA methylation status on the second CpG island of ckβ promoter, HepG2 cells were subjected to a DNA demethylating agent (5-Azacytidine, 5-Aza) and a hypermethylating agent (budesonide). Result showed that 5-Aza induce demethylation effect at -714 site as shown by the reduce mC amount and increase amount of C, but hmC (hydroxymethylcytosine) level was not affected. In contrast with its hypermethylating roles, budesonide induced demethylation effect at -714 site as shown by the reduce amount of mC and increase amount of C and resulted in significant increase of hmC level. Analysis at -769 and -899 sites revealed that 5-Aza treatment reduce the amount of mC level, whereas an increase of mC level was seen with budesonide treatment.

**Conclusion:** In conclusion, this study demonstrated all the three sites on the second CpG island of ckβ promoter were methylated, and can be regulated through epigenetic alteration.

**Supervisor:**
Associate Professor Dr Feu Ling Ling
Co-supervisor:
Dr Noor Fattawati Mokhtar

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**ETHNOMEDICAL SURVEY, PHYTOCHEMICALS ANALYSIS AND BIOLOGICAL ACTIVITY OF SELECTED ANTI DIABETIC PLANTS USED BY ABORIGINES IN GUA MUSANG, KELANTAN**

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MSc

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**Introduction:** Diabetes is one of the chronic diseases affecting worldwide population. Presently, there is a growing interest in herbal remedies for diabetic patient. Therefore, an investigation based on ethnomedicine is required in determined alternative approaches to treat diabetics, such as herbal.

**Objectives:** The main objective of this study was to collect ethnomedical of medicinal plants used by aborigines in Gua Musang, Kelantan. The phytochemical, phytounit and biological activities of selected plants were screened.

**Methods:** Ethnomedical information was collected by interviewing the aboriginal households (house-to-house interviews) and traditional healers in the village. The antidiabetic potential of aqueous extract of selected medicinal plants was determined using α-glucosidase and α-amylase inhibition assay. Phytochemical and phytounit were quantitatively determined using standard procedure and antioxidant activities were determined using 2,2’-azinobis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and ferric reducing/antioxidan power (FRAP) assays.

**Results:** The ethnomedical data revealed that 46 plant species were used in treating various types of health problems, from common diseases such as muscle aches and fever to chronic diseases such as diabetes, hypertension and malaria. From the species recorded, three plants used to treat diabetes is Albizia myriophylla, Oxalis barrelieri and Tacca cristata. Therefore, those plants were term as antidiabetic plants in this study. All extracts were found to have significant antidiabetic activities. A. myriophylla extract showed the highest inhibitory activity against α-amylase in which IC50 15.05 µg/ml, while O. barrelieri showed the highest inhibitory activity against α-glucosidase in which IC50 52.40 µg/ml. O. barrelieri showed the highest phenolic content (64.30 ± 1.50 mg GAE/g), flavonoid content (19.29 ± 2.90 mg CE/g), tannin (42.59 ± 10.23 mg TAE/g), alkaloid (3.27 ± 0.33%), fat (1.47 ± 0.60%) and protein (10.61 ± 0.72%) while T. cristata showed the highest content of saponin (7.17 ± 1.15), ash (10.25 ± 0.15%), carbohydrate (53.51 ± 0.94%) and gross energy (240.93 ± 1.74 kcal/100g). Mineral analysis indicates higher concentrations of magnesium, sodium, calcium, mangan, ferum and zinc in T. cristata while higher concentrations of potassium and phosphorus in O. barrelieri. O. barrelieri extract also had the highest antioxidant activities in ABTS, DPPH and FRAP assays in which 205.95 µmol Trolox/g, 110.41 µmol Trolox/g and 229.93 µmol Trolox/g were obtained, respectively. Further, the most potent extract which is O. barrelieri was subjected to functional beverage development for antidiabetic study in rat model. The results revealed that the O. barrelieri juice showed blood glucose lowering effects in STZ-induced diabetic rats.

**Conclusion:** A. myriophylla, O. barrelieri and T. cristata were found to possess significant in vitro antidiabetic and antioxidant activities. Besides that, O. barrelieri juice showed antioxidant potential in rat animal models.

**Supervisor:**
Dr Norfarizan Hanoon binti Noor Azmi
Co-supervisors:
Dr Hasmah binti Abdullah
Introduction: Water concoction of Quercus infectoria galls (QIG) or Manjakani has long been used by the Malay old folks for various purposes. However, there is still scarce of scientific literature pertaining to the safety of QIG particularly during pregnancy.

Objectives: The present study was aimed to evaluate the potential toxicity of QIG aqueous extract on the fertility and embryonic development in female Sprague Dawley rats.

Materials and Methods: Experimental rats were administered with QIG aqueous extract daily via oral gavage at doses of 0 (control), 125, 250, 500 or 1000 mg/kg/day started from pre-mating period, continuously until gestation day 16 and sacrificed on day 20 of pregnancy.

Results: QIG extract did not cause any mortality, adverse health status or abnormal behavioural changes in all rats. Additionally, there were consistent trend on the maternal body weights (MBW), corrected maternal body weight (CMBW) and maternal weight gain among all groups of animals. The mean length of oestrous cycles was not statistically affected but revealed irregular patterns in some animals upon administration of QIG. The pregnancy parameters including pregnancy index, total number of corpora lutea, number of implantation sites, reproductive organ weights, percentages of pre-implantation loss and post-implantation death revealed no deleterious effects in all groups. All foetuses exhibited normal physical characteristics with the absence of congenital malformation.

Conclusion: Administration of QIG extract of up to 1000 mg/kg/day produced no selective toxicity on the fertility, pregnancy and foetal developmental parameters, except for the moderate changes in oestrous cyclicity data of rats which require further detailed evaluation. Thus, the no observed adverse effect level (NOAEL) detected in this study is 125 mg/kg/day.

Supervisor:  
Dr Wan Ezumi Mohd Fuad  
Co-supervisors:  
Associate Professor Dr Hasmah Abdullah

Molecular Blood Group Typing in Banjar, Jawa, Mandailing and Kelantan Malays in Peninsular Malaysia

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MSc (DNA Profiling)

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Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Blood group antigens are immunogenic proteins or glycoproteins located on the exofacial surface membrane of red blood cells. These blood group antigens are encoded by polymorphic genes and their frequencies are highly variable between unrelated populations.

Objectives: The present study was aimed to genotype ABO, Rhesus, Kell, Kidd and Duffy blood group loci in DNA samples collected from four Malay subethnic groups in Peninsular Malaysia. The blood group data collected from these Malay subethnic groups were then subjected to population and health analyses.

Methods: Blood samples were collected with informed consent from 120 healthy and unrelated individuals. These individuals belong to 4 Malay subethnic groups; Banjar (n = 30), Jawa (n = 30), Mandailing (n = 30) and Kelantan (n = 30) Malays. The QIAamp blood DNA Mini Kit (Qiagen®, Hilden, Germany) was used to extract genomic DNA from the blood samples. The extracted DNA samples were typed for ABO, Rhesus, Kidd and Duffy loci using the BAGene ABO-Type variant, RH-Type and KKD DNA-SSP typing kits (BAG Health Care GmbH, Lich, Germany).

Results: The A, B, O, DCce, K-k-, Jk(a+b-) and Fy(a+b-) were recorded to be the most frequent blood group phenotypes in the four Malay subethnic groups. A principle coordinate (PCO) plot constructed using ABO and Rhesus phenotype frequencies demonstrated a close genetic relationship between the four Malay sub-ethnic groups and other Southeast Asia (SEA) populations and thus reflects the shared genetic history among these populations. The probability values suggested a lower chance of an exact match even if blood was randomly transfused between a recipient and donor of similar ethnicity. Therefore, blood transfusion centre should provide blood that match at multiple blood group systems and recruit increase number of donors especially those with rare blood group profile.

Conclusion: The present study has successfully genotyped ABO, Rhesus, Kidd and Duffy blood group loci in Banjar, Jawa, Mandailing and Kelantan Malays using the PCR-SSP technique. Findings from the present study support a common ancestry of Malay subethnic groups and other Austronesian populations. The present study also endorse the application of molecular approaches for blood grouping and highlight the important of supply blood that is match at multiple blood group systems to patients.

Supervisor:  
Dr Edinur Hisham Atan  
Co-supervisors:  
Dr Zafarina Zainuddin  
Mr S Panneerchelvam
**Title:** Nursing Educational Intervention Towards the Reduction of Work-Related Low Back Pain and Enhancement of Body Mechanics Skills in the Nursing Profession

**Abstract:**

Nurses experience the highest incidence of work-related back injuries, a serious and costly problem that affects their profession. In Malaysia, interventional studies regarding work-related lower back pain (LBP) among nurses are limited. Thus, facts related to this problem are only inferred from regional and international studies.

**Objectives:**

This study attempts to assess the effectiveness of an educational program in reducing work-related LBP, improving nurses’ quality of life and nursing skills in using proper and correct body mechanics among Malaysian nurses working at the Hospital Universiti Sains Malaysia (Hospital USM).

**Methods:**

Pre-test and post-test intervention based on the LBP education and clinical training were selected as method of intervention. Cross-sectional study was selected in the first part of this study using a stratified random sample of nurses selected from all wards in Hospital USM. The sample size was 300 nurses with 100% response rate. Randomised control trial study was selected for second part with sample of 35 in each control and intervention group.

**Results:**

The study showed that the prevalence of work-related LBP was 51%. Mean score of pain severity in the first part of the study was 2.47. Multiple logistic regression analysis show that three factors that contributed to the occurrence of work-related LBP which are nurses who assume poor body posture at work, who do not have work organisation and who perceive poor health status. These groups of nurses increased odds of having work-related LBP at 243.571, 32.058, and 0.066 times respectively. Fifteen of 31 nurses from intervention group did not experience LBP after the educational module, whereas 32 of 35 nurses from the control group experienced LBP (P = 0.008). One-way repeated-measures ANOVA showed a significant decreased in LBP severity (P < 0.001), time experienced of LBP (P<0.001) in the intervention group after the interventional module. Also, a significant decrease in pain duration was found between control and intervention group after interventional module. A significant improvement in various physical and psychological factors was found between the control and intervention group after the intervention.

**Conclusion:**

This study promoted the effectiveness of educational program module with clinical training into basic nursing education and health care practices in reducing the incidence of work-related LBP among nurses.

**Supervisor:**

Dr Soon Lean Keng

**Co-supervisors:**

Dr Moh Nazhari Mohd Nawi

Professor Wan Aasim Wan Adnan

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**Title:** Mechanics Skills in the Nursing Profession

**Abstract:**

Dr Soon Lean Keng

**Supervisor:**

Dr Moh Nazhari Mohd Nawi

**Co-supervisors:**

Dr Moh Nazhari Mohd Nawi

Professor Wan Aasim Wan Adnan

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**Title:** Nursing Educational Intervention

**Abstract:**

Rohani binti Mamat

**Master in Nursing**

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**Introduction:**

World Health Organization and the United Nations Children’s Fund recommend that all infants should be exclusively breastfed immediately within one hour post-delivery. The decision and practices to early breastfeeding initiation are influenced by many contributing factors. It is well recognized that poor knowledge and practice of early breastfeeding is pronounced among mothers.

**Objectives:**

This research was conducted to assess the predictive factors of knowledge, perceptions, practices and experiences of primipara mothers as well as its related factors towards early breastfeeding initiation (BFI) in University Kebangsaan Malaysia Medical Centre (UKMMC).

**Methods:**

A cross-sectional study was conducted and two hundred and fifteen primiparas (n = 215) were recruited via purposive sampling at the postnatal wards, UKMMC. Data was collected using a self-administered questionnaires adapted from the Newborn Feeding Ability and Breastfeeding Initiation Practices. The questionnaires included socio-demographic data, knowledge, perceptions, practices and experiences of mothers towards early BFI.

**Results:**

Half of the respondents (46.5%) had higher knowledge of early initiation of breastfeeding, however the majority of respondents (52.1%) revealed gaining insufficient support from the midwives to assist them in initiating early breastfeeding. There was a significant association between higher education and level of knowledge on early BFI (P = 0.001). Additionally, there is a significant association between higher income and level of perceptions of early BFI (P = 0.015).

**Conclusion:**

It can be concluded that the mothers are still lacking in knowledge of early BFI while intentions regarding breastfeeding their infants is poor. Hence, the BFI training program for both mothers and midwives is needed. This study suggests that systematic assessment of knowledge and practice of ten steps successful breastfeeding among midwives in UKMMC should be established to evaluate their competency in supporting mothers to breastfeed infants.

**Supervisor:**

Dr Soon Lean Keng

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**Title:** Knowledge, Perception and Experiences of Primipara Mothers on Early Breast Feeding Initiation in Universiti Kebangsaan Malaysia Medical Center

**Abstract:**

Rohani binti Mamat

**Master in Nursing**

School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan

**Introduction:**

Low back pain (LBP) is a significant condition that affects their profession. In Malaysia, educational program module with clinical training into basic nursing education and health care practices in reducing the incidence of work-related LBP among nurses.

**Objectives:**

It is well recognized that poor knowledge and practice of early breastfeeding is pronounced among mothers.

**Methods:**

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**Supervisor:**

Dr Soon Lean Keng

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**Title:** Knowledge, Perception and Experiences of Primipara Mothers on Early Breast Feeding Initiation in Universiti Kebangsaan Malaysia Medical Center

**Abstract:**

**Supervisor:**

Dr Moh Nazhari Mohd Nawi

**Co-supervisors:**

Dr Moh Nazhari Mohd Nawi

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**Title:** Knowledge, Perception and Experiences of Primipara Mothers on Early Breast Feeding Initiation in Universiti Kebangsaan Malaysia Medical Center

**Abstract:**

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**PhD**

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**Introduction:**

Nurses experience the highest incidence of work-related back injuries, a serious and costly problem that affects their profession. In Malaysia, interventional studies regarding work-related lower back pain (LBP) among nurses are limited. Thus, facts related to this problem are only inferred from regional and international studies.

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**Conclusion:**

This study promoted the effectiveness of educational program module with clinical training into basic nursing education and health care practices in reducing the incidence of work-related LBP among nurses.

**Supervisor:**

Dr Moh Nazhari Mohd Nawi

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