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Abstract

The cytoskeleton is a dynamic network of actin filaments, intermediate filaments, and microtubules, which is the cytoplasmic scaffold to give the cell shape and motility. The spatial architecture rearranges to enable a variety of cellular function including cytokinesis, organelle distribution, and intracellular trafficking. Here, we would like to elucidate spatial architecture of actin cytoskeleton from molecular to cell levels using recent cryo-EM and AFM data as well as a result of conventional freeze-etching EM, that is important to understand the molecular basis of cytoskeleton dynamics and its impact on cell biology. Unroofing method was used for preparation to observe the membrane cytoskeleton in cryo-EM, freeze-etching EM and AFM. In practice, an improved unroofing method has enabled us to panoramically view the membrane cytoskeleton in its native state with extremely high contrast in cryo-electron microscopy. Many actin filaments and microtubules were clearly observed on the cytoplasmic surface of the cell membrane. Actin filaments extended in all directions in a smooth contour with little branching. Microtubules spread out as far as 3μ m or more while slightly winding in fresh state. Upon fixation, the microtubules became straight and fragmented. Cryo-EM also revealed a network of smooth ER beneath the cell membrane in native cells. Such architecture was also confirmed by freeze-etching EM and AFM. Unroofing was useful for immuno-labelling in cryo-EM as well. Antibody-labelled IQGAP1, one of the effector proteins facilitating the formation of actin filament networks, was localized alongside actin filaments.

Keywords: Actin filaments; cryo-electron microscopy; atomic force microscopy
Identification of transcription-related structures in the cell nucleus

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Abstract

In this talk, I will address technical issues of imaging intra-nuclear processes as well as latest challenges with identification of lipid functions in gene transcription. While fluorescent microscopy allows for simultaneous detection of multiple antigens, the electron microscopy (EM) sensitive immunodetection is limited to only two antigens. I will summarize the current possibilities of single molecule visualization inside of cells and tissues, and discuss future needs of researches in biomedicine. In order to overcome the current limitations of immunodetection, we prepared a set of novel nanoparticles (NPs) which fulfill several criteria: size in the frame of 5-12 nm, small size distribution, good contrast and stability in the electron microscope, stability of colloidal solution during conjugation, and surface properties allowing for conjugation with antibodies. With the use of novel NPs, various combinations with commercial gold NPs can be made to obtain a set for simultaneous labelling. For the first time in ultrastructural histochemistry, up to five molecular targets can be identified simultaneously. Using our previously developed tools of spatial statistics one could then map the regions of distribution of multiple molecular targets within the cell, as well as to analyse a high number of individual molecular interactions. Also, we characterized some of the critical steps during (cryo)sample preparation in order to achieve the best preservation of both ultrastructure and antigen in cells. Phosphatidylinositol 4,5-bisphosphate (PIP2) functions in the cell nucleus as a regulator involved in chromatin remodelling, transcription, and splicing. Since its involvement in RNA polymerase II (Pol II) transcription is still little understood, we studied the role of nuclear PIP2 in the organization of Pol II transcription complexes, and based on the results obtained, we hypothesize that PIP2 islets, due to their heterogeneous multi-component nature, have a role in the spatial formation and maintenance of transcription factories and they thus participate in nuclear organization.

Keywords: Immunodetection, nanoparticles (NPs); phosphatidylinositol 4,5-bisphosphate (PIP2)
Mechanistic insights into Y-Box binding protein-1 (YB-1) mediated breast cancer metastasis

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Abstract

Y-box binding protein-1 (YB-1) is an evolutionary conserved master regulator of transcription and translation, frequently found to be overexpressed in malignancies. This presentation will focus on some of the mechanistic pathways involved in YB-1 mediated metastasis in breast cancer, in particular via Coronin-1C, a member of the Coronin family of actin binding proteins that regulate remodeling of the cytoskeleton. Correlation of YB-1 with breast cancer metastasis was first analyzed using clinical tissue samples, in vivo and in vitro experimentation. Global gene expression profiling and stable isotope labeling by amino acid in cell culture (SILAC) whole proteome profiling were then carried out in YB-1 knocked down MDA-MB-231 cancer cells. Subsequently, phenotypic assays were performed on a putative YB-1 downstream target CORO1C in MDA-MB-231 and Hs578T breast cancer cells. YB-1 was observed to be significantly associated with positive axillary lymph node status in clinical breast cancer samples. A significant increase in metastatic lung deposits in an in vivo metastasis model was seen in YB-1 overexpressing breast cancer cells. Silencing of YB-1 protein inhibited cell migration and invasion in MDA-MB-231 cells. Global gene expression profiling and quantitative whole proteome profiling, identified CORO1C as a potential downstream target of YB-1 in MDA-MB-231 cells. Luciferase reporter assay revealed that CORO1C is an indirect downstream target of YB-1, whereby subsequent siCORO1C knockdown in MDA-MB-231 cells and Hs578T cells were observed to reduce cell migration. The novel association of YB-1 with Coronin-1C may offer new insight and targeted therapeutic approach to combat breast cancer metastasis.

Keywords: YB-1 protein; coronin-1C; metastasis; breast cancer

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Vitamin C as an adjuvant for the 2nd generation of anti-cancer treatment of lung and colorectal cancers

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Abstract

Three generations for anti-cancer treatment have been known. The 1st generation is a conventional one and shows direct toxicity to the cancer cells and some normal cells so that the fatal adverse effects are their weakest point. The 2nd one is targeted one, developed via blocking small molecules or mAb raised for the substance involved in checkpoint of carcinogenic process so that the fatal adverse effects are much less common. Nevertheless, the critical limitations of the 2nd generation are a low efficacy of treatment and eventual induction of resistance. The 3rd one is an immunotherapy, which is most ideal, but stands just in the starting point. Vitamin C has been reported to have anti-cancer effects by themselves especially in high doses more than 1 mM. Here, adjuvant effects of vitamin C for the 2nd generation anti-cancer treatment will be introduced. Firstly, three non-small cell lung cancer (NSCLC) cell lines were treated with gefitinib (Iressa, blocking small molecule) alone vs combination of gefitinib and vitamin C. The survival rates of NSCLC cell lines were 85.6±5.4% with gefitinib alone vs. 52.7±7.3% with combination therapy (P=0.046). The downregulation of intracellular signaling cascades, including EGFR, Akt, Erk and Stat3, was also observed in combination therapy. Secondly, human colon cancer cells with a mutant KRAS, which are usually resistant to cetuximab (blocking mAb) treatment, showed an increased chemo-sensitivity in athymic nude mice to the combination treatment of cetuximab and high dose of vitamin C in sodium dependent vitamin C transporter (SVCT)-2 dependent manner. These two experiments provide some scientific clues to resolve the problems such as resistance induction and low treatment efficacy, occurring in the 2nd generation anti-cancer treatment by using relatively high dose of vitamin C as an adjuvant.

Keywords: The 2nd generation anti-cancer treatment; resistance; adjuvant; vitamin C
ZIKV alters DNA methylation status of hippo signaling pathway genes in human neural progenitor cells

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Abstract

Zika virus (ZIKV) infection during pregnancy is associated with increased incidence of congenital microcephaly in offspring of infected mothers. Diverse mechanisms including DNA methylation control normal brain size, which is achieved by controlled proliferation and differentiation of neural stem cells during embryogenesis. It is known that ZIKV shows tropism for neural progenitors; however the mechanistic link between ZIKV infection and congenital microcephaly remains unidentified. Human neural progenitor cells (hNPCs) were infected with Asian strain of ZIKV and subjected to high throughput DNA methylome analysis. Altered methylation of several CpG sites in hNPCs exposed to ZIKV was identified, suggesting that ZIKV altered the methylome in hNPCs. Pathway analysis identified that genes from the Hippo signaling pathway such as WWTR1 (also known as TAZ), GLI2, SMAD3, WNT9B, PAR6, DLG2, NF2 and RASFF1A were differentially methylated in hNPCs infected with ZIKV. The Hippo signaling pathway is a key determinant of organ size and regulates diverse cellular process including centrosome function, that is implicated in cell fate determination. Further analysis revealed that ZIKV infection altered the expression of several CRM genes and reduced the expression of stem cell markers NESTIN, SOX2 and MUSASHI1 in hNPCs, suggesting that ZIKV alters the fate of hNPCs. In summary, our results reveal that ZIKV alters the DNA methylation status of genes from the Hippo signaling pathway leading to downregulation of CRM genes in hNPCs, thereby resulting in perturbed fate of hNPCs. These results highlight the molecular basis underlying ZIKA induced microcephaly.

Keywords: Zika virus; congenital microcephaly; DNA methylome; TAZ gene
Telomeres and telomerase in ageing and cancer

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Abstract

Telomeres are essentially tandem repeats of any DNA sequence that are present at the ends of chromosomes. Their biology has been an enigmatic one, involving various molecules interacting dynamically in an evolutionarily well-trimmed fashion. Telomeres invariably associate with specialised protein complexes that envelop it, also regulating access of the ends to legitimate enzymes involved in telomere metabolism. Loss of telomere equilibrium and associated chromosome-genomic instability might effectively promote tumour progression. Telomere function may have contrasting roles: inducing replicative senescence and promoting tumourigenesis and these roles may vary between cell types depending on the expression of the enzyme telomerase, the level of mutations induced, and efficiency/deficiency of related DNA repair pathways. An alternative telomere maintenance mechanism has been identified in mouse embryonic stem cells lacking telomerase RNA unit with amplification of non-telomeric sequences adjacent to existing short stretches of telomere repeats. Because the maintenance of telomere function is crucial for genomic stability, recent studies provided new insights into the telomere rapid deletion process and into the mechanisms of chromosome instability, ageing and tumour formation. Telomere biology is emerging to appear ever more complex than previously envisaged, with the continual discovery of more molecules and interplays at the telomeres.

Keywords: Telomeres; telomerase; ageing; cancer
The high-voltage electron microscopy in biomedical researches

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Abstract

Transmission electron microscopy (TEM) provides high resolution images, which are useful in studying ultrastructure of cells and tissues. We have to use very thin section about 60~100 nm thickness due to poor penetration power of the conventional TEM at 100 kV. To overcome this limitation, TEMs using higher accelerating voltage have been developed. TEMs can be categorized into conventional TEM, intermediate TEM, high voltage TEM (HVEM), and ultrahigh voltage TEM according to their accelerating voltage. HVEM using 500~1,000 kV has an enough penetration power to observe thick specimen up to 3~4 μm, which is useful understanding 3 dimensional configuration of the cell and tissue. I would like to introduce HVEM system and its some biological studies undertaken at my laboratory from mitochondria analyses in the whole mount grid sample to nervous tissue imaging to understand neural plasticity. Recent advance in HVEM hardware would provide more detailed information in future studies.

Keywords: HVEM; mitochondria; neural plasticity; learning and memory
Proceedings

From conventional electron microscopy to novel x-ray microscopy

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Abstract

In order to see to believe, microscopes have been widely used for research and development in various fields. Depending on the need for magnification and purposes, optical microscope, scanning electron microscope, transmission electron microscope, and scanning probing microscope can be employed. In my research interests, scanning and transmission electron microscopy has been commonly used, previously in materials science and engineering and currently in biomaterial, bioengineering, and biology. In addition to in-house type conventional electron microscopy, novel X-ray microscopy using synchrotron radiation is also employed in some of my studies to reveal better research outcome. Using synchrotron X-ray microscopy, differences of element in valence state, cross-sectional view through sample thickness, and even a 3D reconstructed model can be observed. In the present talk, researches incorporated with various characterization techniques, especially electron microscopy and synchrotron X-ray microscopy will be addressed.

Keywords: X-ray microscopy; synchrotron radiation; microscopes
Analytical possibilities of nuclear microscope with highly focused proton

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Abstract

The novelty of fast ion beam is its ability to traverse through matter with the well-defined straight path having very little lateral spread, for example, 2MeV proton beam can penetrate 5µm into polymer PMMA with only 25nm lateral spreading. This unique characteristic has been demonstrated in various applications, especially in the field of biological tissue and single whole cell imaging using the Nuclear Microscopy (NM) facility in the Center for Ion Beam Applications, National University of Singapore. Probing into thin biological tissue and single whole cell without slicing the cell into thin sections at high spatial resolutions is not easy: conventional light microscope can’t resolve features smaller than 250nm due to the limitation of fundamental diffraction limits of visible light; electron microscopy has nanometer resolution but is limited to viewing only surface features because electrons are easily scattered. The NM facility in the National University of Singapore has the state-of-art performance of probing thin tissue section with 0.5~1µm and single whole cells with sub-100nm fine proton beam and alpha beam. It has also been used for material modification to create different nano and micron structures for various applications. My talk will include an introduction to the state-of-art NM facility operated using fast ions (protons and alpha particles) and its various applications by using the combination of nuclear techniques such as scanning transmission ion microscopy (STIM) for providing detailed density contrast images of thin biological tissue and single whole cells with and without NanoParticles, Rutherford Backscattering Spectrometry (RBS) for determining the depth of NanoParticles from the surface to inside the cells, proton-induced X-ray emission (PIXE) for trace elemental mapping and quantification measurements, proton-induced fluorescence (PIF) for imaging the fluorescence dye to which cell-nucleic acid or other cell organelles might be sensitive. We believe the NM facility in CIBA has excellent potential to extract more information from the thin section of biological materials and inside single whole cells in a much more unique way, and will be complementary to other types of microscopy widely used in the imaging world.

Keywords: Nuclear; microscope; proton beam
Advances in microscopy - single particle electron microscopy

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Abstract

Cryo-electron microscopy (cryo-EM) together with computerized single-particle analysis is proving indispensable for studying the structure of large macromolecules at near-atomic resolution. Advances in cryo-electron tomography have also enabled the study of macromolecules within their natural environments. These studies will become even more useful, when technologies for improving the signal-to-noise ratio such as direct electron detectors and phase plates become widely available. Automated image acquisition has significantly reduced the time and effort required to determine the structures of macromolecular assemblies. Knowledge of 3D structure of macromolecules will facilitate a deeper understanding of their function, and could also help in the designing of small molecules that interact with these macromolecules, as potential therapeutic agents.

Keywords: Single particle electron microscopy; cryo-EM; automated image
Spoilt for choice: Selecting the right microscopy technique for your research and applying it

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A fundamental step for both researchers and core-facility staff is choosing the most appropriate microscope and technique for a given research project. This decision is typically made through knowledge and understanding of the research project and imaging capabilities available. Here I will present a method for formalising this process and enabling a researcher to be led to which technique(s) are most suitable – designed for the suite of equipment housed in the Microscopy Platform at A*STAR in Singapore, but adaptable to other facilities. I will subsequently provide examples of how live-cell imaging and 3D-SIM superresolution microscopy are being applied within the Microscopy Platform for research programs in skin research, genetic diseases and stem cell biology. Specific examples will be introduced to how these advanced microscopy techniques have proven critical in furthering the understanding of conditions ranging from fertility to skin fragility. Live-cell imaging allows us to observe and quantitate the dynamic processes of cells as they grow, migrate, interact with one another and progress through the cell cycle. By contrast fixed cell imaging produces only single time-point snapshots, which must be pieced together to tell the whole story. In addition, the sample preparation, especially the fixation and labelling, may introduce artifacts. By overcoming the challenges of maintaining the cells in culture and, importantly, ‘happy’ on the microscope stage we gain a greater insight into the biological processes in question. We combine live-cell imaging with various microscopy techniques, such as phase contrast, DIC, widefield fluorescence and optical sectioning techniques (deconvolution and confocal). 3D structured illumination microscopy (3D-SIM) enables microscopy at finer resolution than can be achieved with standard optical microscopes. Abbe’s diffraction limit had meant that, until recently, lateral resolution had been limited to ~250nm at best. 3D-SIM relies on exciting fluorescent samples with a structured pattern, moved through multiple angles and phase positions. As the structured illumination interacts with the structures inherent in the sample moiré fringes containing higher frequency information are produced. Subsequent computational processing and reconstruction allows a resolution of ~120nm to be attained.

Keywords: Super resolution microscopy; 3D-SIM; Live-Cell imaging; laminopathies
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Anatomy education? What’s Next!

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Abstract

Modify, adapt, redesign, innovate! All terms that define what has been and is occurring in medical education in United States Medical Schools. New trends include active learning, increased integration, contextual learning, team-based and interprofessional approaches, and longitudinal formats. What’s the anatomist to do? Do what we have always done – move forward and be a leader. Assess ALL teaching options available and develop a program that fits your school’s curricular philosophy and moves your students towards their professional goals.

Keywords: Medical education; anatomy; innovation
The use of technology to promote active learning in the gross anatomy laboratory

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A gross anatomy laboratory is a small group, a highly interactive learning environment that offers abundant opportunities for active learning, wherein students engage in activities that promote higher-order thinking. There is accumulating evidence to show that active learning can increase student scores and reduce the failure rate. However, there are barriers to implementing active learning, including large class, anxiety on the loss of control, and students’ failure to engage in high-order learning. Potentially, technology can help teachers and students overcome at least some of these difficulties. In this talk, the evolution of the use of technology in the gross anatomy laboratory at The University of Hong Kong will be traced. Initially, the TV broadcasting system enables efficient delivery of learning materials from the sources (often the teachers) to the students. The use of video streaming technology, using inexpensive equipment, allows much more flexibility on where and what the teachers can demonstrate and share with the students in the sizeable gross anatomy laboratory. Teachers can broadcast well-dissected structures and anatomical variations from any cadavers to the whole class. However, true bidirectional interactivities become possible after the installation of tablets, one per dissection team of students, and the use of educational software for collaborative exercises. For example, in problem-oriented dissection, students need to work together to solve the clinical problem on their cadavers. The use of a collaborative app allows the student teams to supply individual teams’ solutions, via their tablets, to a cloud document and to comment on one another’s answers. In the newly developed peer-support system for dissection, the app iClass (developed by the e-Learning Technology Development Laboratory of The University of Hong Kong) is used to foster collaboration. Students can use the tablets to produce short videos, showcasing structures that they have successfully found. The videos produced by different teams are made available to all teams through the iClass platform, from which they can view what other teams have found and how they did it. They can rate these videos and give one another feedback. Our experience shows that the use of technology can overcome some of the barriers to active learning in the gross anatomy laboratory, and can even create new learning opportunities not possible before, by enabling bidirectional interactivities, not just between teachers and students, but even among the students.

Keywords: Technology; active learning; anatomy laboratory

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Thoughts on avenues for future research into anatomical sciences education

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Abstract

The landscape of higher education has changed over the last decade with regard to student expectations and ways of learning. UK Universities will soon be judged not only on the quality of research done, but also on the quality of education provided by way of the teaching excellence framework. Relating this to anatomy education, there is a need to understand why what we do works to enable our teaching practises to be evidence based and strengthened by pedagogical theory. Some existing educational theories can be used to explain the success of many current methods used to teach anatomy, though this link is not often made explicit in published literature. Given it is known that students treat the learning of anatomy very differently when compared to other subjects, the need to develop anatomy specific pedagogies is likely. Further considerations are research into students’ metacognition when studying anatomical sciences. There has been an increasing move to incorporate playful learning methods e.g. clay modelling & painting 3D printed models, into anatomy teaching, mostly with success. These methods enable students to gauge their level of understanding and demonstrates to them what further learning is required to fully understand the topic. Further research into helping students understand how to measure their progress in anatomical learning i.e. their metacognition, is needed.

Keywords: Anatomical science education; playful learning methods; metacognition

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Anatomy teaching, the way forward: Applications of smartphone and mobile apps for teaching and learning anatomy and biomedical sciences

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Mobile technologies and smartphones have enabled mobile learning to happen 24/7 at any time and any location convenient to the learners. It is estimated that 2.53 billion smartphone users in the world are using mobile devices as the primary means of accessing the internet and online contents. With this increased penetration and use of smartphone and mobile apps, mobile learning with apps are gaining popularity in tertiary education. This session will provide an overview of the use of mobile apps as a tool for Anatomy and biomedical sciences. A check into App store and Play store in April 2018 revealed more than 600 mobile apps that are available for Anatomy education; many of them are free with useful content like “Essential Skeleton 4”. This workshop and presentation will feature some of the top 10 mobile apps used in Anatomy education and share some of these apps like “Essential Anatomy 5” and “Essential Atlas of Animated Anatomy and Physiology” used in our student learning. We also used newer smartphone educational tools such as kahoot, socrative, mentimeter and padlet, which are very useful in monitoring our student learning. The workshop will also feature a live demo on the use of Kahoot and mentimeter for the participants to try out. The session will also cover some of the barriers and factors that can determine the use of smartphones and mobile app as a useful tool for teaching and learning Anatomy and biomedical sciences.

Keywords: Anatomy teaching, mobile apps, learning
Alloferon as a novel therapeutic agent for cancer, viral infection and inflammation

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Alloferon is a novel immunomodulatory peptide originally isolated from infected insects. It has anti-viral and anti-cancer effects as well as anti-inflammatory effect. However, its specific action mechanisms are largely unknown. For this reason, we investigated the role of alloferon and its specific anti-viral, anti-cancer and anti-inflammatory mechanisms. First, we have found that alloferon effectively regulated the life cycle of influenza virus and human papilloma virus. Second, alloferon directly suppresses proliferation of several kinds of cancer cell including lymphoma, pancreatic cancer and non-small cell lung cancer. It also shows cancer killing effect through the activation of NK and CTL. Third, alloferon more effectively regulates the development and progress of acute and chronic inflammatory disease, such as inflammatory bowel disease, contact dermatitis, UVB-induced skin inflammation and pulmonary fibrosis via the down-regulating production of inflammatory cytokines, IL-1α/β, IL-6, IL-8 and TNF-α. Taken together, alloferon has pleiotropic role in cancer and several kinds of inflammatory disease via direct regulation of disease-related factors and immune activation.

Keywords: Alloferon; immune modulation; anti-cancer effect; anti-viral effect; anti-inflammatory effect
Translational control through the formation of messenger RNPs

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Abstract

Structures of messenger ribonucleoproteins (mRNPs), which represent mRNA-protein complexes consisting of mRNA and associating proteins, are dynamic. mRNP remodelling events are likely to occur during various post-transcriptional steps of gene expression. The Y-box proteins are multifunctional DNA- and RNA-binding proteins involved in various aspects of gene regulation. The founding member of the Y-box protein family, YB-1, is a principal component of cytoplasmic mRNPs of somatic cells and is required for optimal translation. YB-1 also functions as a transcription factor which activates the transcription of genes involved in cell proliferation or tumour progression. YB-1 is highly expressed in a variety of human cancers, and its nuclear localisation is associated with tumour malignancy. Thus, regulating the amount or activity of YB-1 in cells would have an impact on the synthesis and cytoplasmic fate of mRNA in the cytoplasm. We previously identified YBAP1/C1qbp as a Y-box protein-associated acidic protein, which relieves the translational repression induced by YB-1 in vitro. We show that YB-1 stabilises a reporter mRNA and overexpression of YBAP1 partially abrogates this effect in cells. We also find that YBAP1 removes YB-1 from mRNPs in cell extracts, suggesting that YBAP1 can act as a mRNP remodelling factor. Our data lend further support for the integral role of the Y-box proteins in modulating mRNP structure and function.

Keywords: Translation; Mrnp; YB-1

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Anti-inflammatory potential of crude extracts from the sea cucumber, *Holothuria Scabra*, in LPS stimulated RAW264.7 macrophages

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Abstract

Sea cucumber, *Holothuria scabra*, has long been used as a food and traditional medicine. It has been reported that the extracts from the sea cucumber can regulate various cellular and biological functions. However, the role of *H. scabra* in anti-inflammation and its molecular regulation remains unclear. In this study, we investigated the anti-inflammatory effect of the crude extracts from *H. scabra* by using lipopolysaccharide (LPS) to induce an inflammatory response in the RAW264.7 macrophage cell line. The results indicated the crude extract from the whole body of *H. scabra* (WBHS) was non-toxic to the cell and could inhibit pro-inflammatory cytokines synthesis, mainly nitric oxide (NO), interleukin-1β (IL-1β), and tumour necrosis factor-α (TNF-α). In addition, WBHS was able to downregulate phosphorylation of JNK. These findings indicated that WBHS might have the potential to be developed as a natural anti-inflammatory agent.

Keywords: *Holothuria Scabra*; inflammation; RAW264.7; sea cucumber
Autophagy in alcohol-induced organ damage: Lessons from animal models

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Abstract

Excessive alcohol intake may induce damage to almost all organs of the body. Autophagy is a cytoprotective pathway for isolation of pro-apoptotic cellular components into autophagic vesicles, and clearance via lysosome. Autophagy could be selective for specific organelle such as mitochondria (mitophagy). Here we investigated the involvement of autophagy in acute ethanol-induced organ damage. Adult male Wistar rats were injected intraperitoneally either with 5 g/kg ethanol or phosphate buffer saline (for the control group) and sacrificed within 24 hours after injection. Testes and livers were removed and processed for light and electron microscopic studies. Parts of these organs were frozen for Western blot. Ethanol-induced testicular damage was investigated in cultured Sertoli cells (SCs). Compared to low levels in control groups, upregulation of autophagy proteins (LC3, pan cathepsins, LAMP-2) and mitophagy marker (PINK1) were predominantly detected in hepatocytes and SCs of ethanol-treated rats (ETRs) using immunohistochemistry, immunofluorescence and Western blot. Electron microscopy showed the accumulation of autophagic vacuoles in the above-mentioned cells of ETRs, which appeared normal and non-apoptotic. Cultured ethanol-treated SCs exhibited high autophagic activity, but their viability was reduced on blocking autophagy. Enhanced autophagic response in various organs of ETRs may be an adaptive mechanism for survival and may have therapeutic implications in alcohol-related diseases.

Keywords: Autophagy; ethanol; oxidative stress; mitophagy; lipophagy; LC3; PINK1.
Molecular and morphological evidence of hepatotoxicity after silver nanoparticle exposure: An updated systematic review, in silico, and ultrastructure investigation

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Abstract

Silver nanoparticles (AgNPs) have been widely used in a variety of applications in innovative development; consequently, people are more exposed to this particle. Growing concern about toxicity from AgNP exposure has attracted greater attention, while questions about nanosilver-responsive genes and consequences for human health remain unanswered. By considering early detection and prevention of nanotoxicology at the genetic level, this study aimed to identify 1) changes in gene expression levels that could be potential indicators for AgNP toxicity and 2) morphological phenotypes correlating to the toxicity of HepG2 cells. To detect possible nanosilver-responsive genes in xenogenic targeted organs, a comprehensive systematic literature review of changes in gene expression in HepG2 cells after AgNP exposure and in silico method, connection up- and down-regulation expression analysis of microarrays (CU-DREAM) were performed. In addition, cells were extracted and processed for transmission electron microscopy to examine ultrastructural alterations. From the Gene Expression Omnibus (GEO) Series database, we selected genes that were up-regulated in AgNPs, but down-regulated in silver ion exposed cells, as nanosilver-responsive genes. HepG2 cells in the AgNP-treated group showed distinct ultrastructural alterations. Our results suggested potential correlations between gene level responses and ultrastructural alterations triggered by AgNPs. Collectively, our findings representing gene data after AgNPs exposure provide insight into the assessment and prediction of toxicity from nanosilver exposure.

Keywords: Hepatotoxicity; Silver Nanoparticles; Ultrastructure

Keywords: X-ray microscopy; synchrotron radiation; microscopes
Application of neurotransplantation for treating Parkinson's disease: Allograft and xenograft

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Abstract

Parkinson’s disease (PD) is a neurodegenerative disorder characterised by pathological features such as severe loss of dopaminergic neurons in the substantia nigra pars compacta. Currently, for the cell therapy of PD, there are several potential resources of dopaminergic neurons which are derived from different stem cells. Progenitor cells of VM have been grafted into the striatum of PD patient to treat PD remarkable effects. However, the use of the fetus also provokes severe ethic issues leading to the limited clinical application. Use of dopaminergic neurons derived from the embryonic stem cells from the inner cell mass of blastocyst for treating PD has raised concern as transforming into cancer cells in the grafted area. Instead, somatic cells are transformed into induced pluripotent stem cells by transfection of Yamanaka 4 factors and then differentiated to dopaminergic neurons. Nevertheless, tumorigenesis of these cells has been reported. Although neural stem cells derived from a fetus or adult brain could be used to treat PD, such approaches are clinically feasible and practical. Xenograft using porcine embryos represents an ideal alternative for treatment of PD, which may have less ethical issues. The survival rate of grafted dopaminergic neurons has been an issue to overcome. New strategies for the treatment of PD using xenograft are described.

Keywords: Neurotransplantation; parkinson’s disease; allograft; xenograft
Faster extension for a predetermined associational collateral of a cortical dual-projection neuron

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Abstract

Direct connections between different cortical areas have been shown to be important for sensorimotor integration. Developmental processes of these connections, however, have not been fully understood. By using a subtype-specific promoter and our new sparse labeling method combined with tissue clearing of flat-mounted cortices, we visualized a population of layer 2/3 neurons in the mouse primary somatosensory area (S1) that had axons projecting to a distant ipsilateral target (associational projection) as well as to the contralateral hemisphere (callosal projection). After the main axonal shaft crossed the midline, collateral branches projecting to the ipsilateral hemisphere emerged. Only a few among these collateral branches reached the distant target areas, such as the primary motor area (M1) and the secondary somatosensory area (S2). These far-reaching branches grew at a higher rate than those projecting to the local targets, suggesting that a branch or two might be selected at a very early stage to target an area distant from the original area of the soma.

Keywords: Association; collateral; cortex; development; longitudinal fasciculus
Docosahexaenoic acid (DHA) metabolism induces pyroptosis in BV2 microglial cells

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Abstract

DHA is one of the most abundant fatty acid in the brain, largely present in stores of membrane phospholipids. It is readily released by the action of phospholipase A2 and is known to induce anti-inflammatory and neurotrophic effects. It is not thought to contribute to pro-inflammatory processes in the brain. An immortalized murine microglia cell line (BV-2) was used to evaluate the effect of DHA on neuroinflammatory cells. Cells were cultured as adherent monolayers under standard culture conditions, and treated with DHA and with inhibitors of DHA metabolism. Cell responses were measured by morphological analysis, qRT-PCR, and biochemical analysis of caspase activity. Pretreatment of BV-2 cells with low concentrations of DHA attenuates lipopolysaccharide-mediated inflammatory cytokine gene expression. However, higher (but still physiologically relevant) concentrations of DHA induce profound cell swelling and a reduction of viability. This is accompanied by an increase in the expression of inflammatory cytokine and lipooxygenase genes, and by the activation of caspase 1 activity, indicating that cells were undergoing a pro-inflammatory cell death program known as pyroptosis. This process could be attenuated by inhibition of 12-lipooxygenase (12-LOX, Alox12e), but not by inhibition of 5-LOX or 15-LOX. Although DHA is known to mediate anti-inflammatory processes, we show that high, but physiologically relevant concentrations of DHA activate pro-inflammatory cell death pathways in microglial cells, resulting in the release of inflammatory cytokines. This is mediated not by DHA itself, but by one or more bioactive metabolites.

Keywords: Neuroinflammation; pyroptosis; microglia; docosahexaenoic acid (DHA); lipooxygenase,
Interaction with donors’ families added empathy cultivation to gross anatomy teaching

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Abstract

Medical students learn structures by dissecting human bodies. Dissection started on criminals, grave-robbed bodies, the unclaimed, and the donated in some countries nowadays. Donating bodies for medical education remains controversial. The lack of transparency in treating donated bodies and the reluctance of the surviving families to see the bodies subjected to cuts and dismantling are factors alienating the public from the donation. Dissection of the unwilled without the person’s consent is inconsistent with modern medical, educational emphasis on humanity and empathy. Tzu-Chi University pioneered a humanistic-based curriculum that used only willed bodies starting from its first dissection class in 1996. Donors are silent mentors or altruistic role models to the students. Students connect to the donation by visiting surviving families and learning about the donors before classes. Families and students joined the ceremonies at the beginning and end of the dissection. Interaction leads students to appreciate better the donation and in-depth introspection to be caring professionals in the future. It, in addition, comforts surviving families and helps to quail public resistance to body donation. In short, gross anatomy program can be designed to include interaction to connect the learners to the altruistic philosophy of body donors and surviving families. This brings anatomy teaching beyond simply learning body structures to a new level of significance, i.e. empathy cultivation, which is a critical aim of modern medical education.

Keywords: Altruistic; cadaver; empathy; humanity; silent mentor

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Body donation in Thailand: Cadavers as teachers

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Abstract

The donation of bodies for medical studies creates an invaluable contribution to the advancement of medical knowledge. The human body is an invaluable and indispensable aid in medical education. A fundamental basis of all medical knowledge relates to a thorough understanding of human anatomy which can be learned only by a study of the human body. Each year in Thailand, thousands of cadavers are needed for teaching medical, dental, and health-related professions students. Currently, most Thai people realise the importance of donating their bodies for purposes of medical education. There are at least two factors that encourage most Thais to donate their bodies. The first factor is that donors attain the highly regarded status of "ajarn yai", the great teacher. This status is conferred in the elaborate dedication ceremonies. The second but most important factor is that the Late King Bhumibol Adulyadej has officially approved the Royal Cremation Ceremony for donors. In addition, donors are guaranteed that their remains will be treated with dignity, respect and anonymity at all times. In Thailand, the cadaver is treated much more like a social person and less like an object. During the course, the cadavers are always referred to as ajarn yai. Paying respect to these teachers is paramount. Students know the name, age, and cause of death of the body they are dissecting. At least two ceremonies must be organised in each Thai medical school, the dedication ceremony some days before the first-course session and the Royal Cremation Ceremony at the end of the course. These ceremonies are important events for faculty members, students and relatives of the deceased. A major symbolic theme is to "make merit" for the spirits of the deceased. The Royal Cremation Ceremony of the dissected bodies culminates in a large procession led by Buddhist monks, in which the students carry their ajarn yai to the cremation building. At the ceremony, the dissected bodies are burnt by the Royal Torch brought in by the officers from the Bureau of the Royal Household. The body donation in Thailand reflects all these aspects of the status accorded to the donors and the ceremonial acknowledgement of the donors and their families.

Keywords: Ajarn Yai; dissection; human body; royal cremation ceremony.
Proceedings

Donation as silent teacher at Siriraj Hospital in Thailand

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Abstract

At Siriraj hospital, over the past nine years in 2009-2017, the average number of donated body per year is 7824, 33.67% male, 66.33% female and body gathered to Anatomy department per year is 323; 58.39% male, 41.60% female. Like other medical schools, the increase in body donation is related to the strong incentive in Thai society with respect and believe in the valuable of silent teacher “Ajarn Yai” for the medical and research studies. Seventy percent of silent teachers are prepared as embalming bodies for the second year medical students in Siriraj hospital, affiliated institutes, and other requests from medical schools. About 30% of silent teachers are prepared as soft and fresh cadavers for postgraduate, residency teaching, research and specialist training program in Siriraj training and educational centre (SiTEC). In addition, the bone collection, anomaly organs and particular dissection like fine art are also developed from the silent teachers for a demonstration in the well establishment of Congdon’s anatomical museum which is opened for public visiting and some learning and teaching projects from different fields of study rather than medical study. Using the public media like TV, the faculty informs the student activities in Royal cremation ceremony, what has been done and how important it concerns the silent teacher in the museum and the training workshops in SiTEC. Therefore, the increased body donation with expresses the most profound gratitude and appreciation for the spiritual intention of those who donate their bodies for the medical study is still very appreciated in Thai society.

Keywords: Body donation; cadaver; anatomy

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Proceedings

Zinc oxide nanoparticles induced oxidative stress in Drosophila fruit fly

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Abstract

Zinc oxide nanoparticles (ZnO NPs) have been widely used in various consumer products. However, there has been an increasing number of reports on the toxicity of ZnO NPs. We investigated the toxicological profiles of ZnO NPs using the fruit fly Drosophila as an in vivo model. The physico-chemical characteristics of ZnO NPs used in this study were determined by various tools such as Transmission Electron Microscope (TEM) and ZnO NPs were administered into flies through the oral route. ZnO NPs were observed to be spherical in shape, with an average hydrodynamic size of ~70 nm and a surface charge of +5.8 mV. Furthermore, TEM study showed that ZnO NPs ingested were accumulated in the gut. Notably, ZnO NP exposure caused a dose-dependent decrease in egg-to-adult survivorship and a significant delay in development, but their toxic effects were found to be size-dependent. Interestingly, considerable amounts of ROS were generated upon ZnO NP exposure in the gut, as evidenced by a drastic increase in Dihydroethidium (DHE) staining, suggesting that oxidative stress could be responsible for decreased viability and delayed development caused by ZnO NPs. Nrf2 (nuclear factor E2-related factor 2) plays an important cellular sensor for oxidative stress. Thus, we posited that ZnO NP-mediated cytotoxicity would be even more severe when the activity of Nrf2 or detoxifying enzyme such as Superoxide Dismutase 2 (SOD2) is downregulated. In support of this, removal of one copy of CncC (the Drosophila homolog of human Nrf2) or SOD2 further increased ZnONP-induced toxicity. Taken together, this study suggests that ZnO NPs can induce a significant oxidative stress-related toxicity in Drosophila. More extensive studies would be needed to verify the safety issues related to increased usage of ZnO NPs by consumers.

Keywords: Zinc oxide nanoparticles; Drosophila, reactive oxygen species; antioxidant

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Proceedings

Genetic and epigenetic profiles of BDNF gene are associated with METH dependence and METH-dependent psychosis

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Abstract

Methamphetamine (METH) is an addictive psychostimulant. Intense abuse of METH induces psychotic symptoms similar to those of schizophrenia. Brain-derived neurotrophic factor (BDNF) is a neurotrophin which has roles in modulation of synaptic neurotransmission. Alterations of BDNF protein have been reported in many neuropsychiatric diseases including drug addiction. Therefore, genetic or epigenetic processes of BDNF, encoding for BDNF protein, might be involved in METH dependence. The objective of this study was to investigate the association of a functional genetic polymorphism and DNA methylation of BDNF in METH dependence and METH-dependent psychosis. The rs6265 was genotyped in 202 male Thai samples (102 controls, 53 METH with psychosis and 47 METH without psychosis) using real-time PCR-high resolution melting (HRM). The DNA methylation of BDNF and Line-1, a measure of global DNA methylation, was examined in prefrontal cortex (PFC), hippocampus and striatum of METH-administered rats using bisulfite pyro-sequencing. The results showed that GG genotype of BDNF rs6265 is significantly associated with vulnerability for METH dependence and METH-dependent psychosis in the Thai sample. Moreover, DNA methylation of BDNF, but not Line1, was significantly different in PFC and hippocampus of METH dependent rats compared to controls. The results suggested that both genetic variability and DNA methylation of BDNF gene are associated with METH dependence and METH-dependent psychosis, an effect that may relate to alterations of BDNF expression resulting in neuropathological changes underlying METH dependence and METH dependent psychosis.

Keywords: Methamphetamine; Brain-Derived Neurotrophic Factor; DNA methylation; psychosis; gene polymorphism

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Association of ARID1A tumor suppressor protein expression in renal cell carcinoma and patient pathological outcome

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Abstract

Renal cell carcinoma (RCC) is one of the most common lethal cancer due to it shows the evidence of resistance to chemotherapeutic treatment. The novel tumor suppressor protein named AT-rich interactive domain 1A (ARID1A) has been reported in several organs of cancer that the ARID1A lost in these cancer organs especially in gynecologic cancers. However, few studies have been conducted on the expression of ARID1A in RCC. The expression of ARID1A protein was investigated in twenty-six RCC human tissues using immunohistochemistry, and find out the association of ARID1A expression and pathological outcome of patients. The results showed that ARID1A showed loss of its expression in Fuhrman nuclear grade II of clear cell type and chromophobe types (80.2%). Moreover, loss of ARID1A expression trended to associate with following outcome including male (65.4%), age > 60 years-old (46.2%), right side of RCC (53.8%), greatest dimension of tumor > 7 cm. (42.3%), Fuhrman nuclear grade II (38.5%), and hemorrhage (73.1%). These results are consistent with previous studies that loss of ARID1A expression associated with high grade of RCC and poor prognostic values. From these results, the loss of ARID1A tumor suppressor protein may involve in the progression of RCC particular in male and older age patients. However, a large number of RCC sample should be investigated in further study.

Keywords: Renal cell carcinoma; ARID1A protein; immunohistochemistry
A preliminary study of the collagen density and thickness of the unexposed skins in Thai cadavers

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Abstract

The skin thickness and collagen fiber content in dermis of the skin are important in dermatologic research and treatment. It has been reported that both skin thickness and dermal collagen fiber are different in area, age and gender. However, the histological study of the skin in Thai people has less information. Therefore, we aimed to perform the preliminary study of the skin thickness and dermal collagen fiber density in Thai male and female. Scalp and skin of thigh from 71 years old male and 53 years old female cadavers were collected. The paraffin embedding blocks (FFPE) of all samples were performed. The paraffin sections were stained by hematoxylin and eosin (H&E) dyes for epidermal and dermal thickness measurement. The dermal collagen density was studied using Masson’s trichrome staining. We found that the epidermis of both unexposed areas of male was thinner than those of female especially at the dermal-epidermal junction. The result of the study is consistent to the previous study which has been reported that the thickness of epidermis and dermis were significantly decreased in patients aged >60 years old and gradual reduction in the dermal-epidermal junction. However, the dermal thickness and dermal collagen density of female cadaver were thinner and less than in those of male. These findings suggest that the onset of menopause in >50 years old women has an effect on estrogen decline and may cause the reduction of collagen production resulting in decreasing dermal thickness in female cadaver.

Keywords: Epidermal thickness; dermal thickness; dermal collagen; unexposed skin

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Anticancer effect of chrysoeriol in glioma

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Abstract

Flavonoids, a widely distributed category of plant metabolites, are known to exhibit a wide range of biological functions. In the present study, the possible anticancer effects of chrysoeriol, a flavone extracted from the Thai endemic herb Phyllanthus niruri, were investigated by using the in vitro cytotoxicity assays. We evaluated the effect of chrysoeriol on the induction of autophagy and investigated whether chrysoeriol-induced autophagy exerts the anti-proliferative effect in rat C6 glioma cells. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The expression of cell proliferation- and autophagy-related proteins, PTEN, beclin-1 and LC3II/LC3I, were analyzed by western blotting and immunocytochemistry. Chrysoeriol treatment significantly reduced the viability of rat C6 glioma cells. Cell proliferation- and autophagy-related proteins, including PTEN, beclin-1 and LC3-II/LC3I ratio, were significant increased by chrysoeriol treatment. These results suggest that chrysoeriol exerts anticancer effects through the suppression of cell proliferation and the induction of cellular autophagy in rat C6 glioma cells. Chrysoeriol demonstrates anticancer effects under in vitro conditions and might be a potential anticancer agent for the treatment of glioma.

Keywords: Chrysoeriol; anticancer; glioma; PTEN; Beclin-1; LC3II/LC3I Ratio
Anatomical variation of maxillary sinus floor in patient undergoing surgical dental treatment

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Abstract

To achieve the best dental treatment for dental patients undergoing maxillary surgery or implants, we have to be sure about the anatomical variation of maxillary sinus floor. Focusing about this issue will make our intervention safe and manipulation more successful, too. This study aims to appraise efficacy of panoramic radiograph in evaluation of the level of maxillary sinus floor among Malay population in Kuantan city which included full dentate, partial and complete edentulous. 364 panoramic radiographs were collected from unite of radiology for patients who attended dental polyclinic in Faculty of Dentistry, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia. Those data classified based on the absence or presence of the teeth into 118 full dentate, 192 complete edentulous and 54 partially edentulous. Measurement of the distance was done either between upper posterior teeth and maxillary sinus floor or residual ridge and maxillary sinus floor. Finally, study the effect of age and gender on this measuring distance. The analysis showed that distance not affected by age and gender in full dentate while in complete and partially edentulous the distance decreased by increasing age which was more noticeable in males more than females. Good quality panoramic radiograph is helpful in assessment of the level of maxillary sinus floor in full dentate, complete and partial edentulous, less radiation, less cost in comparison with other type of images such as CT scan where the ionizing radiation is much more than conventional radiographs.

Keywords: Anatomy; distance; edentulous; maxillary sinus; panoramic radiograph

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Screening for lipids and polyunsaturated fatty acids from the economic seaweed

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Abstract

Marine algae are important sources of high beneficial compound, including sulfated polysaccharides, antioxidants, and bioactive secondary metabolites. Lipids also play a role in cellular function, inflammation, antitumor, antibacterial, and reproduction through fatty acid molecules especially PUFA and HUFAs. Two abundantly found algae near the west coast of the gulf of Thailand i.e. Sea lettuce (Ulva rigida) and Blubber weed (Solieria robusta) extracts were studied using Thin-layer chromatography (TLC) and Gas chromatography mass spectrometry (GC-MS). In this study we showed the composition of phospholipids, especially PCs and total fatty acids (FAs) in three fractions of U. rigida and S. robusta extracted by ethanol, chloroform-methanol, and ethyl acetate. PCs is present in all fractions but reaching maximum in chloroform-methanol extract of U. rigida. Analysis by GC-MS indicated that the major FAs present in all fractions were composed of 14:0, 16:0, 17:0, 18:0, 16:1, 18:1, 18:2, 20:1, 20:2, 20:4, 20:5, and 22:6. The chloroform-methanol extract of U. rigida and S. robusta contained the largest amounts of these FAs. This knowledge will be useful in formulating supplementary food containing appropriate amounts of fatty acids, PUFA, and HUFA from marine algae.

Keywords: Ulva rigida; Solieria robusta; marine algae; lipids; fatty acids; GC-MS

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Characterization of stem cells isolated from human dental pulp

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Abstract

Dental pulp stem cells from permanent (DPSC) and deciduous teeth (SHED) are adult mesenchymal stem cells that may function as an alternative source to replace a "gold standard" bone marrow stem cells for cellular therapy. The characterization of the DPSC and SHED are important in manipulating their potential for tissue regenerative purpose. This study aims to characterize DPSC and SHED in terms of morphology, molecular stemness, proliferation and osteoblast differentiation. Dental pulp was extracted from human permanent and deciduous teeth using an enzymatic digestion method and was cultured until passage 3. Morphology of the cells was recorded with cellB software and molecular characterization was conducted using RT-PCR. The proliferation rate of DPSC and SHED were assessed by 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay and osteoblast differentiation potential was determined by using alkaline phosphatase (ALP) assay. Both types of cells exhibited fibroblast-like morphology at passage 3 with molecular marker expression pattern of CD73+, Cd105+, and CD146+. The proliferation rate of SHED was significantly higher than DPSC (p<0.05). This study also showed that the specific ALP activity was significantly higher in SHED compare to DPSC. Both cells capable to manifest fibroblast-like morphology, expressing selected mesenchymal stem cells marker with SHED showed higher proliferation and osteoblast capability compared to DPSC.

Keywords: Deciduous teeth; permanent teeth; fibroblast; osteoblast; proliferation; stemness

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PIWI protein enhances neuronal differentiation of human embryonic carcinoma cells

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Abstract

piRNAs are ~26-32 nucleotide small non-coding RNAs that bind specifically to the PIWI subfamily of argonaute proteins. Some pilot work has suggested that PIWI has a vital role not only in germline stem cells but also in somatic stem cells. Though recent evidence have revealed the presence of PIWI/piRNA in brain, the exact role of PIWI/piRNA complex in the nervous system is still unknown. Hence, we aim to investigate the role of PIWI protein in neuronal differentiation of human pluripotent stem cells. The human NT2 cells (pluripotent human embryonal carcinoma cells) are a well characterized cell line that resembles human embryonic stem cells (hESC) and is able to differentiate into mature neurons in the presence of retinoic acid (RA) with the loss of tumorigenicity. We studied the expression profile of individual PIWI homolog proteins in RA-mediated differentiation of human NT2 cells to neurons. During the course of differentiation, we found that a particular PIWI homolog is gradually increasing. With the knockdown of this homolog, we found that the embryonic stem cell markers were upregulated, and neuronal differentiation markers were suppressed in the presence of RA. Interestingly, we also observed that the PIWI homolog interacts with epigenetic regulators to modulate the expression of various developmental genes. By understanding the role of small RNAs and their associated proteins in neuronal development, we will be able to enhance neuronal differentiation of stem cells and greatly benefit the patients with neurodegenerative diseases.

Keywords: PIWI; neuronal differentiation; retinoic acid; embryonic stem cells

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High glucose alters the DNA methylation of genes involved in axon guidance mechanism in human neural progenitor cells

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Abstract

Diabetes during pregnancy has been shown to be a major risk factor for susceptibility to early life metabolic and cognitive impairments in the offspring. It is well established that maternal diabetes alters the morphological and global expression of genes related to neural tube development in mouse embryos. Lately, epigenetic mechanisms such as DNA methylation, histone modifications and regulation of non-coding RNAs, were found to be globally altered in mouse neural stem cells exposed to high glucose, but the underlying gene-specific epigenetic mechanisms are yet to be revealed. The present study investigated altered gene-specific DNA methylation patterns in human neural progenitor cells (hNPCs) exposed to high glucose, as the DNA methylation modifications regulate the transcriptome. The gene-specific DNA methylation alterations were estimated using high-throughput illumina human MethylationEPIC array in hNPCs exposed to both physiological (5mM) and high glucose (40mM) concentrations. The results revealed altered DNA methylation pattern in genes involved in several pathways including axon guidance pathway in hNPCs exposed to high glucose. Interestingly, DNA hypomethylation was found in gene body region of Slit1, a key regulator of axon guidance, in hNPCs exposed to high glucose. Interestingly, DNA hypomethylation was found in gene body region of Slit1, a key regulator of axon guidance, in hNPCs exposed to high glucose. Further analysis revealed a decrease in protein expression of Slit-1, Robo2 (an interacting partner of SLIT1), and Srgap1 in guiding axons during neural tube development, in hNPCs exposed to high glucose. In addition, Slit1 knockdown in hNPCs also down regulates the gene expression of Robo2 and Srgap1 suggesting altered Slit1-Robo2 signaling axis. This finding suggests that high glucose alters the gene-specific DNA methylation patterns of genes critical for axon guidance mechanism which can lead to neural tube defects and cognitive impairments in the newborn. This epigenetic information can be used for identifying potential therapeutic targets to treat hyperglycemia-related birth anomalies.

Keywords: DNA methylation; human neural progenitor cells; hyperglycemia
Ultrastructural effect of *Polygonum minus* essential oil on cisplatin-induced hepatotoxicity in *Sprague dawley* rats

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Abstract

Cisplatin is a chemotherapeutic agent widely used in treating cancers. However, its usage is restricted due to its toxic effect on liver, which is seen in approximately 36% of cancer patients receiving cisplatin treatment. *Polygonum minus* has high level of antioxidants, as well as terpenoid and phenolic compounds which contains several bioactive properties. The objective of this study is to investigate the ultrastructural effect of *Polygonum minus* essential oil (PmEO) on cisplatin-induced hepatotoxicity in male rats. Thirty male rats were divided into five different groups such as control (C), 10 mg/kg cisplatin-treated (CP), PmEO 100 mg/kg (PmEO100), PmEO 200 mg/kg (PmEO200), PmEO 400 mg/kg (PmEO400). PmEO was given orally for 14 days. All animals except in the control group were injected a single dose of cisplatin (10mg/kg) intraperitoneally on day 15 and were sacrificed on day 18. Liver specimens were prepared for electron microscope study. Examination of specimens by electron microscopic study revealed that cisplatin caused hepatotoxicity exhibited by diminished glycogen content, increased number of mitochondria, vesicular dilated rough endoplasmic reticulum and nuclear changes in cisplatin-treated rats. However, these changes were minimized in the PmEO100 treated groups. Therefore, we concluded that the PmEO100 has protective effect against cisplatin-induced hepatotoxicity.

Keywords: Chemotherapeutic agent; liver toxicity; *Polygonum minus* essential oil, cisplatin, liver enzyme
Comparative anti-tyrosinase and antimicrobial activities of seed coat extracts between four varieties of sweet tamarind

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Abstract

Tamarind is a traditional herb having medical therapeutic effects. The antimicrobial and anti-tyrosinase properties of tamarind extract have been presented by various investigators whereas these abilities of different types and sources of tamarind’s extract have not been well reported. Difference types of tamarinds may have a difference seed contents and antimicrobial and anti-tyrosinase characters. Hence, the main aim of this study is to compare the antimicrobial and anti-tyrosinase activities of ethanolic seed coat extracts between 4 varieties of sweet tamarinds. Sweet tamarinds (Seethong, Intapalum, Srichompoo and Prakaithong) from Phetchabun of Thailand were tested for antimicrobial and anti-tyrosinase activities. After Dopachrome test, it was found that IC50 of tyrosinase inhibition of Seethong, Intapalum, Srichompoo and Prakaithong seed coat extracts (5, 10, 25, 50 µg/ml) was 16.6, 16.7, 17, 18 µg/ml, consequently. That was almost equal to IC50 of tyrosinase inhibition of Kojic acid (16.4 µg/ml). The antimicrobial potential of seed coat extracts (10 µg/ml) was screened against four bacteria (Escherichia coli, Staphylococcus aureus, Pseudomonas solanacearum E.F. smith and Acinetobacter baumannii) using disk diffusion test. All extracts had antibacterial activities but less than ampicillin and kanamycin. We can conclude that (1) Anti-tyrosinase activity of 4 sweet tamarind coat extracts was not significantly different and similar to kojic acid and (2) Anti-microbial activity of 4 sweet tamarind coat extracts was not significantly different and less than ampicillin and kanamycin.

Keywords: Sweet tamarind; anti-Tyrosinase; antimicrobial
The anti-oxidant capacity of black glutinous rice bran extract and the potential role in the inhibition of kidney stone formation in vitro

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Abstract

Growth and aggregation of calcium oxalate (CaOx) crystal cause a serious kidney stone disease. Anti-oxidative substance administration might interfere the formation of CaOx nidus and lead to the reduction of kidney stone pathology. Therefore, this study needed to investigate the effect of a high anti-oxidative substance, local strain black glutinous rice bran extract, on the CaOx crystal formation inhibition. The black glutinous rice bran (BGB) was extracted with ethanol, evaporated, and dried. The anthocyanin and total phenolic contents were determined. The anti-oxidative capacities were elucidated by DPPH and FRAP methods. After the incubation of BGB extract with CaOx crystal, the number, size and aggregation form of the crystals were counted. BGB extract contained high amount of anthocyanin and total phenolic content. The extract could reduce the DPPH radical and Fe3+. The number, size and aggregation form of CaOx monohydrate crystal were decreased by incubation with BGB extract, while CaOx dihydrate was increased. These results indicated that BGB extract, which exhibited anti-oxidative capacity, could inhibit the formation of CaOx crystal in vitro. This study is the first reveal of anti-nephrolithiasis of black glutinous rice bran, which could be developed for the kidney stone prevention strategy in the future.

Keywords: Kidney stone; black glutinous rice bran; anti-oxidation; calcium oxalate crystal

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Quantitative proteome profiling of Y-Box binding protein-1 (YB-1) overexpressing breast cancer cells identifies STAT3 as a downstream target

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Abstract

Y-box binding protein 1 (YB-1) is a protein involved in many fundamental cellular processes such as mediating chemoresistance, enhancing cell proliferation and inducing metastatic spread in many human malignancies. YB-1 protein was first overexpressed in the MCF7 breast cancer cells (MCF7-YB-1). Subsequently, MCF7-YB-1 cells were grown in stable isotope labelling with amino acids in cell culture (SILAC) medium and whole proteome profiling was performed. Next, transcription factor analysis with MetaCore bioinformatics software using the differentially upregulated proteins identified from the whole proteome was done. To validate the clinical relevance of the selected target, Gene expression-based Outcome for Breast cancer Online (GOBO) database analysis was then carried out. Mass spectrometry based SILAC whole proteome profiling identified 121 upregulated proteins and 120 downregulated proteins in the MCF-7-YB-1 cells. Transcription factor analysis revealed STAT3 as one of the top potential downstream transcription factors of YB-1. Subsequent STAT3 promoter luciferase assay showed an enhanced luciferase signal, suggesting that STAT3 is a downstream target of YB-1. Multivariate analysis of STAT3 in the GOBO database demonstrated that STAT3 correlates with grade 3 tumours and axillary lymph node involvement. Our results show that YB-1 could potentially affect breast cancer progression via STAT3.

Keywords: YB-1 Protein; STAT3; SILAC; breast cancer

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Performance of MC3T3-E1 cells in 2-dimensional flask

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Abstract

MC3T3-E1 cells are progenitor cells that are extracted from mouse calvaria which is from neurocrest origin. It is also known as precursor of osteoblast. MC3T3-E1 cells can only differentiate into osteoblasts; a characteristic of progenitor cells. The objectives are to characterize the morphology, proliferation rate of MC3T3-E1 and analyze the differentiation potential of cell culture into osteoblast. MC3T3-E1 were analyzed in term of morphology using cell B software. Proliferation rate of MC3T3-E1 cells were assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium (MTT) assay. Biochemical analysis, using alkaline phosphate (ALP assay), was done to determine osteoblastic differentiation of these cells. Morphology of the cells presented as mononucleated, fibroblast-like with extended cytoplasmic projection. The cell viability assay showed that the cells maintained their growth rate and the amount of proliferation was increased gradually. The result of ALP assay when compared between MC3T3-E1 in complete medium and MC3T3-E1 that are induced with osteogenic medium showed a significant difference from day 9 until day 21(p<0.05; n=3). MC3T3-E1 was shown to be successfully proliferated and differentiated into osteoblasts.

Keywords: MC3T3-E1; Osteoblast; Alkaline phosphate; MTT Assay

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Histological study of mucin expression patterns in Thai colorectal cancer tissues

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Abstract

Colorectal cancer (CRC) is one of the most common cancers worldwide. Mucin has a role as the main constituent of the mucus-protecting layer in gastrointestinal tract. In normal colorectal tissues, the acid and neutral mucins are found. The mucin alteration is the one parameter of histological features in CRC and has been noticed in cancer development and progression. This study was aims to investigate the mucin expression pattern in various histological grades of CRC: well, moderately, and poorly differentiated adenocarcinoma. Fourteen formalin fixed, paraffin embedded (FFPE) CRC tissues were collected from the pathology unit, Sawanpracharak hospital and the procedure was approved by the human ethics committee of (no.16/2560). The histopathological features of CRC tissues were classified by H&E staining. The Periodic Acid Schiff (PAS) and Alcian blue (AB) staining were applied for neutral and acid mucins expressions. We found that neutral mucin expression was likely decreased and acid mucin were predominantly decreased in the adenocarcinoma area comparing with normal area of the same tissue. The acid mucin expression was significantly decreased in poorly differentiated adenocarcinoma grade of CRC (P<0.05). The decreasing of acid mucin in high-grade CRC was also reported in many studies. It has been suggested that acid mucin may involve the cancer progression due to having a role in cell division controlling and tumor growth inhibition. This was the first report about the decreasing of acid and neutral mucins expression pattern. Then, it will be an importance to perform on larger sample size among Thai population.

Keywords: Colorectal cancer; acid mucin; neutral mucin; Alcian Blue staining; periodic acid; Schiff staining.
Proceedings

Pterostilbene delays the progression of premalignant lesions in the lung squamous cell carcinoma mouse model by histopathology evaluation

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Abstract

Lung cancer is one of the most common cancers with adenocarcinoma(AD), and squamous cell carcinoma(SCC) are the major types of lung cancer. Lung cancer has a very high mortality rate due to late diagnosis that leads to the failure of treatment. Therefore, the studies of chemoprevention have gained increasing popularity against treatment to control this disease. Pterostilbene is a natural compound with varies pharmacological activities such as antiproliferative, anti-inflammatory and others. This study was conducted to investigate the chemopreventive effects of pterostilbene on lung SCC in a mouse model. Balb/C female mice at seven weeks of age were randomised into three groups with (n= 6 mice per group) 1 treatment group and 2 control groups. Group 1 was pre-treated with pterostilbene (10mg/kg) before 0.04M N-nitro-tris-chloroethylurea (NTCU) application, group 2 was the carcinogen control treated with NTCU together with corn oil and group 3 as a vehicle control that received 70% acetone and corn oil. Animals in each group received treatment twice a week and at week 24, all animals were sacrificed for histopathology evaluation. At week 24, carcinogen control group 2 developed pre-malignant lesions with high-grade dysplasia as compared to vehicle control group 3 which showed no changes to the epithelial layers. Interestingly, treatment group 1 with pterostilbene demonstrated mild hyperplasia with some areas of bronchial epithelium remained intact and unchanged. Pterostilbene may suppress the premalignant lesions in the development of lung SCC. Thus, additional studies on the chemopreventive effects of pterostilbene are required.

Keywords: Pterostilbene; chemoprevention; respiratory; lung cancer; squamous cell carcinoma

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Cockle shell originated calcium carbonate based nanobiocomposite bone scaffold

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Abstract

The cockle shells from Anadara granosa sp. are typical biocomposites, essentially consisting of 95-99% calcium carbonate crystals in the form of aragonite. The denser nature of the aragonite form of CaCO₃ from cockleshells gives it an added advantage to be incorporated, resolved and then replaced by bones over time. The nanobiocomposite (nCP) scaffolds are prepared using nano cockleshell powder and alginate hydrocolloid solution in a composition ratio of 60:40 (w/v) using lyophilization and cross-linking techniques in custom-made cylindrical molds to produce a three-dimensional scaffold structure. Scanning electron microscope studies have shown that the scaffold has an ideal pore size ranging from 50 to 336 μm. The scaffold also showed favourable mechanical property of 3.4 MPa that was found to range between the strength of a spongy bone structure. In-vitro studies using osteoblast showed excellent cell attachment, proliferation and growth while in a separate study using mice bone-marrow derived stem cells, the possible osteogenic response of the scaffolds was demonstrated with the findings of mineralization of the scaffold surface as well as detection of calcium and phosphate elements. A study comparing the nCP scaffolds with scaffolds prepared with commercial CaCO₃ showed a significant increase in the release of calcium and ALP enzymes in the nCP scaffolds comparatively indicating the naturally occurring CaCO₃ from cockleshells has better potentials to be used as biomaterials for bone tissue engineering applications. In-vivo studies on the nCP scaffold incorporating BMP-2 growth factors implanted subcutaneously in mice showed an equally good performance of the non-growth factor incorporated nCP scaffold indicating its promising properties in supporting osteogenesis without the use of any promoting factors.

Keywords: Nanobiocomposite; bone tissue engineering

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Adenosine receptor (A2A RA) regulates expression of pro-inflammatory mediators in Lipopolysaccharide stimulated BV-2 microglia pre-treated with its antagonist (SCH58216)

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Abstract

Microglia play important roles in the brain development and in disease conditions. They are the primary source of pro-inflammatory mediators including IL-1β, TNF-α, IL-6 and iNOS in brain infection and inflammation. Therefore, manipulating microglia over-activation is considered a potential therapeutic strategy for different neurological diseases. Adenosine is a well-known purine nucleoside produced from the metabolism of adenosine triphosphate (ATP) intracellularly and extracellularly. It interacts with its four receptors (A1, A2A, A2B, A3) to initiate the biological effects. More importantly, expression of adenosine and its receptor has been detected in microglia. This study aims to determine the expression of pro-inflammatory mediators in BV-2 microglia pre-treated with selective antagonist (SCH58216) of A2RA and stimulated with lipopolysaccharide (LPS) to confirm whether adenosine and its receptor are involved in inflammatory reaction of microglia. BV-2 microglia were pre-treated with the antagonist (SCH58216) of A2RA and stimulated with LPS. We showed that the protein expression level of IL-1β and iNOS was significantly decreased. The mRNA level of IL-1β, TNF-α, IL-6 and iNOS was concomitantly decreased when compared with the control. This was coupled with the decrease of production of ROS. Adenosine receptor (A2ARA) regulates the production of pro-inflammatory mediators in LPS stimulated BV-2 microglia pre-treated with its antagonist. Therefore, suppression of adenosine receptor with its specific antagonist may be a potential strategy to attenuate microglia mediated inflammation in different neuropathological diseases.

Keywords: Adenosine Receptor (A2A RA); Proinflammatory Mediators; BV-2 Microglia
Protective effect of oral pterostilbene on ultraviolet B-induced epidermal hyperplasia in Balb/c mice

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Abstract

Ultraviolet radiation (UV) is an environmental human carcinogen. Prolong exposure may lead to hyperpigmentation, erythema, epidermal hyperplasia and skin cancer. Therefore, the development of UVB protective agent is needed in order to reduce the dermal toxicity. The use of natural active compounds such as pterostilbene can be developed as protective treatment as it has high antioxidant, anti-inflammation, antiaging, and anticancer properties. The aim of the study was to evaluate protective effect of oral pterostilbene on ultraviolet B-induced epidermal hyperplasia in BALB/c mice. Twenty-four female BALB/c mice were randomly divided into 4 groups: vehicle control group (n=6 mice), without UVB irradiation and resveratrol administration; exposure group (n=6 mice), irradiated with UVB only; and two treatment groups (n=6 mice), which were irradiated with UVB and treated with 0.02 ml of 10 mg/kg and 20 mg/kg of pterostilbene by oral gavage. This treatment is given for 14 days. UVB was exposed on day 9, 11, 13. The dosage given is 250 mJ/cm² for 3 minutes. On day 15, skin morphology was observed and skinfold thickness was measured using skinfold caliper to evaluate edema. Then, mice were sacrificed to obtain dorsal part of skin for histopathological observation using Hematoxylin and Eosin (H&E) stain. Oral pterostilbene attenuated skin scaling and ulceration in UVB induced mice. The skinfold thickness of both doses of pterostilbene decreased significantly (p<0.05) as compared to UVB irradiated group. H&E staining showed that the treatment groups show less severe epidermal hyperplasia as compared to UVB irradiated group. Oral pterostilbene able to act as photoprotection by reducing the epidermal hyperplasia and edema. Therefore, it has the potential to be developed as natural alternative for photoprotection.

Keywords: Ultraviolet radiation; Pterostilbene; Epidermal hyperplasia; BALB/c Mice

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Reducing osmium tetroxide usage in preparing *Ocimum gratissimum* L. leaf surfaces for scanning electron microscope analysis

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Abstract

Glutaraldehyde and osmium tetroxide are popular fixatives that have been used extensively for sample preparation to preserve cell structure for scanning electron microscope. However, osmium tetroxide is a harmful chemical. The purpose of this study is to optimize the complete fixative condition to maintain leaf tissues and trichomes of *Ocimum gratissimum* L. Samples were fixed overnight in 2.5% glutaraldehyde followed by 1% osmium tetroxide and was compared to only 2.5% glutaraldehyde fixation. The samples fixed with glutaraldehyde and osmium tetroxide showed shrinkage trichomes on both sides of leaf surfaces. The trichomes fixed in 2.5% glutaraldehyde showed 91.55±3.49 percents of complete trichomes on the upper leaf surface and 91.48±3.60 percents on the lower surface. While the samples fixed in glutaraldehyde and osmium tetroxide combination showed 78.29±4.04 and 76.52±3.66 percents of complete trichomes on upper and lower surfaces, respectively. Thus the glutaraldehyde fixation preserved the integrity of trichome better than the double fixation. Therefore, this condition could eliminate the utilization of osmium tetroxide which is highly toxic to users and environments.

*Keywords: Reducing Osmium Tetroxide; Ocimum Gratissimum L.; Scanning Electron Microscope.*
Roles of Slitrk6 in mouse sacral neural crest cells during the development of the enteric nervous system

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Abstract

Hirschsprung’s disease (HSCR) is a congenital gastrointestinal motility disorder characterized by reduction of neurons of the enteric nervous system (ENS) in the distal part of the colon. These enteric neurons are derived from vagal and sacral neural crest cells (NCCs) during embryonic development. The Dominant megacolon (Dom) mouse mutant, which harbours a spontaneous mutation in Sox10, has been used as an animal model of HSCR. However, the molecular events, especially in sacral NCCs, that lead to the neuronal loss in the mutant remain unknown. To find out the relationship between the Sox10 mutation and the neuronal loss in the gut, we performed expression profiling using microarray. Expression of SLIT and NTRK-like family, member 6 (Slitrk6) was down-regulated in sacral NCCs of Dom mice. Luciferase assay revealed direct binding of Sox10 to the Slitrk6 promoter. Knockdown of Slitrk6 in sacral NCCs resulted in reduced proliferation and enhanced neuronal differentiation 4 days after cultured in a differentiation medium. Conversely, over-expression of Slitrk6 in sacral NCCs led to enhanced proliferation but formation of fewer synapses. We found that expression of Slitrk6 was regulated by Sox10 through direct binding to its promoter and that Slitrk6 was able to keep sacral NCCs undifferentiated through enhancing proliferation and inhibiting precocious neuronal differentiation. This study is the first to illustrate the roles of Slitrk6 in sacral NCCs during the ENS development.

Keywords: Hirschsprung’s disease; Slitrk6; Mouse sacral neural crest cells; Microarray
Scanning electron microscopic study of spores of lycophytes and pteridophytes in Singapore

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Abstract

Spores of lycophytes and pteridophytes harbour distinct surface ornamentation which is reportedly unique to the species. Hence, the spore ornamentation may be a useful taxonomic character. However, it is unclear if spores belong to the same genus or family of these two groups of plants in Singapore are built with a common basic architectural design. Very little information is known about the spore morphology of native lycophytes and pteridophytes which have more than 150 known species. Spores are obtained in Singapore from herbarium or freshly collected specimens that are pressed dry then mounted on special stub and gold-coated for examination under the scanning electron microscope (SEM). This study aims to produce an atlas of spore morphology of local lycophytes and pteridophytes. The external morphology of the spores shows numerous sizes and shapes, and may potentially trigger upper respiratory allergic reactions such as asthma, and other related diseases.

Keywords: Lycophytes; Pteridophytes; Spores

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Leaf surface and pollen morphology of a medicinal herb *Limnophila heterophylla* (Roxb.) Benth

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Abstract

*Limnophila heterophylla* (Roxb.) Benth. is an aquatic plant and important perennial herb. It is used for anti-inflammatory in India. It grows in brackish water, rice field and distributes to Thailand, Myanmar, Vietnam, Nepal and Sri Lanka. The aim of this research is to examine the leaf surfaces and pollen morphological characteristics of *L. heterophylla* by scanning electron microscope. The pollens were fixed in glutaraldehyde and osmium tetroxide and were dried with critical point dryer. The leaf samples were subject to a freeze drying method. The morphological characteristics of pollen including size, shape, exine sculpturing pattern and aperture type were observed and described. Leaf surfaces, stomata and trichomes were also studied. The results showed that the leaf surfaces consist of epidermal cells, stomata and glandular trichomes. The number of stomata on lower epidermis (34±9.01/mm²) is more than the upper epidermis (8±2.42/mm²). The pollen is circular in polar view and subprolate in equatorial view. The aperture is tetracolpate. The exine sculpturing is foveolate type. Leaf surface and pollen characteristic are usually specific to the genus and species of plants. Therefore, the information from this study can be used for *L. heterophylla* identification.

Keywords: Leaf surface; Pollen; Limnophila Heterophylla

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Localization of NCAM and PSA-NCAM in subgranular zone of hippocampus

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Abstract

As neural cell adhesion molecules (NCAM) and the polysialylated (PSA)-NCAM have been detected in serum and CSF as the biomarker for treated acute multiple sclerosis and invasive medulloblastoma, respectively. During brain development, NCAM, a glycoprotein localized in the plasma membrane of neural and glial cells which plays a role in myelination, cell to cell interactions, differentiation and synapse formation. Polysialylation can modify NCAM and involve in migration, proliferation, and differentiation of neural cells. This study aimed to investigate the NCAM and PSA-NCAM expression focusing on newly generated neurons in the subgranular zone (SGZ) of dentate in the hippocampus. The localization of NCAM and PSA-NCAM using immunoperoxidase staining was studied in embryonic E17, postnatal P3, P5, P7, P14, compared to mother mice. Western blot analysis was used to quantitate protein expression levels in postnatal mice brain tissue. In SGZ of the hippocampus, NCAM was found on the cell surface of neurons and glia highly in postnatal mice than embryonic mice that might be involved in cell to cell interaction. PSA-NCAM was found in cytoplasm and nucleus of neurons, more densely staining in P7 and P14 but no staining found in E17 might involve loosing of contact for the migratory process. A similar result was confirmed using Western blot analysis of the postnatal brain tissues. NCAM was significantly increased in P5, P7, and P14 when compared with P3. PSA-NCAM in early stage was higher than late and adult stages. In conclusion, the different expression of NCAM and PSA-NCAM found in this study may be reflected by their serum and CFS levels which can indicate the developmental brain defects.

Keywords: NCAM; PSA-NCAM; Subgranular zone
Proceedings

Attenuated TNF-α and IL-1β by sea cucumber (Holothuria scabra) extracts in vascular dementia mice

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Abstract

In order to see to believe, microscopes have been widely used for research and development in various fields. Depending on the need for magnification and purposes, optical microscope, scanning electron microscope, transmission electron microscope, and scanning probing microscope can be employed. In my research interests, scanning and transmission electron microscopy has been commonly used, previously in materials science and engineering and currently in biomaterial, bioengineering, and biology. In addition to in-house type conventional electron microscopy, novel X-ray microscopy using synchrotron radiation is also employed in some of my studies to reveal better research outcome. Using synchrotron X-ray microscopy, differences of element in valence state, cross-sectional view through sample thickness, and even a 3D reconstructed model can be observed. In the present talk, researches incorporated with various characterization techniques, especially electron microscopy and synchrotron X-ray microscopy will be addressed.

Keywords: X-ray microscopy; synchrotron radiation; microscopes

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Hypoxia-induced histone modifications in activated microglia in vitro

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Abstract

Hypoxia in the developing brain is a pathological state resulting in cognitive disabilities and cerebral palsy. During hypoxic condition in the fetal brain, microglial cells, the resident immune cells of the central nervous system, have been shown to get activated and secrete a plethora of pro-inflammatory cytokines that result in neuronal damage. Current evidence indicates that epigenetic mechanism may contribute to the development of hypoxia-ischemic sensitive phenotype in the developing brain in response to the fetal stress. Hence, the present study was conducted to evaluate the role of hypoxia in the epigenetic regulation of inflammatory cytokines in hypoxia-activated microglia. Murine BV2 microglial cells used in this study were subjected to hypoxic condition at 37 degrees, 5% CO2 and 3% O2. Immunofluorescence staining of BV2 cells was performed to analyse the changes in histone marks and inflammatory cytokines in microglia in response to hypoxic conditions. The histone marks, the repressor and activator of transcription, H3K27me3, H3K9ac and H3K4me3, were included in the study. Immunofluorescence staining showed a decreased expression of anti-inflammatory cytokine, IL-10 in microglia and increased expression of TNF alpha, pro-inflammatory cytokines in the microglial cells exposed to hypoxia. The expression of H3K9ac (activation mark) and H3K4me3 (repressor mark) was analysed in microglia exposed to hypoxia. There is an increase in the H3K9ac mark in microglia after 6h and 24h of hypoxia exposure. Concurrently, a decrease in the expression of the H3K27me3 mark was observed in microglia after 6 hours and 24 hours of hypoxia. Microglial activation in response to hypoxia appears to be epigenetically regulated. Further study involving inhibition of microglial activation using pharmacological agents such as HDAC inhibitor and resveratrol will be discussed.

Keywords: Hypoxia; Microglia; Inflammatory markers; Histones; Immunofluorescence
Anti-inflammatory potential of crude extracts from the sea cucumber, *Holothuria scabra*, in LPS stimulated RAW264.7 macrophages

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Abstract

Sea cucumber, *Holothuria scabra*, has long been used as a food and traditional medicine. It has been reported that the extracts from the sea cucumber can regulate various cellular and biological functions. However, the role of *H. scabra* in anti-inflammation and its molecular regulation remains unclear. In this study, we investigated the anti-inflammatory effect of the crude extracts from *H. scabra* by using lipopolysaccharide (LPS) to induce an inflammatory response in the RAW264.7 macrophage cell line. The results indicated the crude extract from the whole body of *H. scabra* (WBHS) was non-toxic to the cell and could inhibit pro-inflammatory cytokines synthesis, mainly nitric oxide (NO), interleukin-1β (IL-1β), and tumour necrosis factor-α (TNF-α). In addition, WBHS was able to downregulate phosphorylation of JNK. These findings indicated that WBHS might have the potential to be developed as a natural anti-inflammatory agent.

Keywords: *Holothuria scabra*; Inflammation; RAW264.7; Sea cucumber
Effects of naringenin on haemodynamic status and lipid profile in fructose-STZ-induced prolonged hyperglycaemia

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Abstract

Diabetes mellitus (DM) is one of the life threatening chronic metabolic disorders which alarms the global population. Cardiomyopathy and premature atherosclerosis due to DM remains the leading cause of death in Malaysia. Naringenin (NG), an active compound found in citrus fruits, was proven to possess antidiabetic, antihypertensive and anti-hyperlipidaemia properties. The aim of this study was to investigate the effects of NG in prolonged fructose-STZ-induced hyperglycaemia. Excessive fructose administration tend to cause type 2 DM. We used twenty four (24) adult male Sprague-Dawley rats which were randomly divided into four (4) groups (n=6); control (C), non-treated DM (D), NG-treated DM (DNG) (50mg/kg), and metformin-treated DM (DMT). Following four (4) weeks of developing hyperglycaemia, treatment was given via oral gavage for five weeks. Blood pressure, random blood glucose and weight were measured during pre- and post-treatments. Serum lipid profile was analysed and compared in between groups. Serum blood glucose and body weight showed no significant difference between experimental groups. Diastolic blood pressure was significantly reduced in the DNG group, however no significant difference in reducing systolic blood pressure was found in DNG group while compared to D group. Data from lipid profile measurement showed reducing triglycerides and LDL levels in DNG group. No differences were observed in total cholesterol and HDL among the experimental groups. NG is believed to revert the haemodynamic changes and the lipid profile level in fructose-STZ induced hyperglycaemia, but further studies are required to confirm the ameliorative effect of NG in type 2 DM.

Keywords: Naringenin; Fructose; Haemodynamic; Hyperglycaemia

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Characterization of various phenotypes of human erythroid progenitors by mouse monoclonal antibodies

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Abstract

Hematopoietic stem cells are isolated from human cord blood, using CD 133 isolation kit. CD 133+ cells are cultured in serum-free medium with cytokines, HSCs can proliferate and differentiate to become erythroid cells. In each stage of erythroblast, no marker specific to erythroid progenitors. We characterise various phenotypes of human erythroblasts stages using monoclonal antibodies from a mouse immunised with human erythrocytes. Four groups of monoclonal antibodies obtained from hybridoma techniques of the mouse immunised with human red blood cells were used in this characterisation. 80-90% positive cells of WK3(Act) and 297A(Act) express on day 5-15. 60-70% positive cells of 328(4C2) NL SUP and 129(3B11) NL SUP express on day 5-9. 4A(1C3) SUP is low expressed in early erythroblast stage and highly expressed (30-40%) in mature cells. While expression of intermediate levels of #328(1C3) Act and 328 No check no changed during culture d5-15. This was the first study showing the characterise various phenotypes of human erythroblastic stages using monoclonal antibodies from a mouse immunised with human erythrocytes. This information will be useful for the separation of various stages of erythroid progenitors in the future.

Keywords: Erythroid cells; Monoclonal antibodies; Cell culture; Flow cytometry

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Neuroprotective effects of chrysoeriol against MPP\(^+\)-induced cell death through changes in P70S6K and 4E-BP1 phosphorylation

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Abstract

1-methyl-4-phenylpyridinium (MPP\(^+\)) is synthetic toxin used in the in vitro study of Parkinson’s disease (PD). It has been reported that the down-regulation of protein synthesis is one of the key mechanisms of MPP\(^+\)-induced cell death. Ribosomal protein S6 kinase (P70S6K) and Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) are essential enzymes in translation and newly synthesis of protein. These proteins are down-regulated in the presence of MPP\(^+\). Chrysoeriol, a flavonoid compound found in tropical plants, exhibits a variety of pharmaceutical activities including antioxidant and anti-inflammatory properties. Recently, we have reported the protective effect of chrysoeriol extracted from Phyllanthus niruri in MPP\(^+\)-induced cell death in SH-SY5Y cells by modulation of pro-apoptotic Bax protein and anti-apoptotic Bcl-2 protein. However, its effect on protein synthesis has not yet been revealed. The cytotoxicity and cell viability in MPP\(^+\)-treated SH-SY5Y cells were performed by MTT assay. The expression of phosphorylation of P70S6K (Thr389) and 4E-BP1 (Thr36) proteins was examined by Western blotting. We found that MPP\(^+\) significantly decreased the phosphorylation of P70S6K and 4E-BP1. Pretreatment with chrysoeriol significantly reversed the effects of MPP\(^+\). The results suggested that chrysoeriol exhibited significant protective effect against MPP\(^+\)-induced cell death via modulation of protein synthesis. The present study supports the notion that chrysoeriol may be a promising neuroprotective molecule for the prevention of neuronal death in brain caused by neurodegenerative disorders such as PD.

Keywords: Chrysoeriol; MPP\(^+\); Neuroprotection; Parkinson’s disease; P70S6K; 4E-BP1
Anticancer effect of chrysoeriol in glioma

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Abstract

Flavonoids, a widely distributed category of plant metabolites, are known to exhibit a wide range of biological functions. In the present study, the possible anticancer effects of chrysoeriol, a flavone extracted from the Thai endemic herb Phyllanthus niruri, were investigated by using the in vitro cytotoxicity assays. We evaluated the effect of chrysoeriol on the induction of autophagy and investigated whether chrysoeriol-induced autophagy exerts the anti-proliferative effect in rat C6 glioma cells. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The expression of cell proliferation- and autophagy-related proteins, PTEN, beclin-1 and LC3II/LC3I, were analysed by western blotting and immunocytocchemistry. Chrysoeriol treatment significantly reduced the viability of rat C6 glioma cells. Cell proliferation- and autophagy-related proteins, including PTEN, beclin-1 and LC3-II/LC3I ratio, were significantly increased by chrysoeriol treatment. These results suggest that chrysoeriol exerts anticancer effects through the suppression of cell proliferation and the induction of cellular autophagy in rat C6 glioma cells. Chrysoeriol demonstrates anticancer effects under in vitro conditions and might be a potential anticancer agent for the treatment of glioma.

Keywords: Chrysoeriol; Anticancer; Glioma; PTEN; Beclin-1; LC3II/LC3I ratio

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Mkp3 is a novel ROS-induced downstream effector that regulates germline stem cell behavior in the Drosophila testis

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Abstract

Reactive oxygen species (ROS) are by-products of oxygen metabolism. Moderate levels of ROS are essential for cell signalling and normal cellular functions such as stem cell maintenance, whereas aberrant ROS levels have been linked to the pathogenesis of many human diseases such as cancer, neurodegeneration and diabetes by damaging macromolecules. Importantly, the downstream molecules of ROS that are involved in such biological processes, including stem cell maintenance, remain poorly understood. Here, the Drosophila testis was utilized as an in vivo model to identify and characterize molecules that play a role in high ROS-mediated germline stem cells (GSCs) differentiation. Affymetrix microarray analysis was conducted, and more than 150 genes were found to be differentially expressed in high ROS-induced GSC differentiation. Remarkably, more than 30% of these genes were uncharacterized signifying that our assay has identified unique genes with a potential role in ROS-mediated GSC differentiation. Mkp3 (Mitogen-activated protein kinase phosphatase 3), an inhibitor of MAPK, was downregulated upon high ROS. We found that knockdown of Mkp3 leads to an increase in ROS levels and causes a loss of GSC by promoting differentiation in the Drosophila testis. Knockdown of Mkp3 also increased the expression of phosphorylated Erk1/2 in the Drosophila testis. Interestingly, overexpression of Mkp3 caused an expansion of GSC-like cells positive for the mitotic marker pH3. Taken together, our results suggest that high-ROS induced GSC differentiation is highly associated with the Mkp3-MAPK/ERK signalling pathway in the Drosophila testis.

Keywords: Mkp3; Reactive Oxygen Species (ROS); Germline stem cell (GSC); Drosophila; Differentiation.
Microscopic observation on cardiac tissue and coronary artery in sudden death cases

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Abstract

Sudden death (SD) is an unexpected natural death occurs within 24hrs with or without the onset of symptom most often used to describe death caused by the cardiac failure. The aim of this study investigates the trend between demographic factors and the histological changes in myocardial tissue and coronary artery in sudden death cases. Medico-legal cases were obtained from the Department of Forensic Hospital Canselor Tuanku Mukhriz (HCTM). Left Ventricle Myocardium tissue and Left Artery Descending (LAD) were collected during the autopsy and undergo microscopic observation. Tissues were graded respectively according to its artery occlusion percentage (Grade 0–4), myocardial infarction and thrombus formation. From medico-legal cases, there were 25 cases of SD with the majority involving men (96%). Age range between 50–59 (36%) turned out to be the highest. For ethnicity, Malays (40%) dominated the cases, whereby the majority of the cases demonstrated body mass index (BMI) range between underweight (56%) and obese (40%). In addition, there were 10 (40%) cases of the coronary artery with atheroma and 15 (60%) cases of myocardial infarction based on histopathological examination. The highest number of cases was grade III with 11 patients (44%) followed by grade IV with five patients (20%). Whereas for myocardial infarction mostly in acute myocardial infarction stage with 14 (56%) cases. Based on the results, we can conclude that there was a strong association between histological changes of the heart and coronary arteries with demographic factors. However further investigation needs to be done to investigate any specific biomarker to predict SD in the future.

Keywords: Atherosclerosis; Autopsy; Myocardial Infarction; Sudden cardiac death; Sudden death.
Phosphoglycerate dehydrogenase enhances proliferation in breast cancer

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Abstract

Phosphoglycerate Dehydrogenase (PHGDH), a rate-limiting enzyme in the serine synthesis pathway, belongs to the family of oxidoreductases. Cancer cell proliferation is known to be dependent on serine metabolism for aerobic glycolysis. Hence, this study was conducted to evaluate the role of PHGDH in regulating breast cancer cell proliferation. The relationship of PHGDH and Proliferating Cell Nuclear Antigen (PCNA), a cell proliferation marker, was first determined by performing immunohistochemical staining of the two proteins in tissue microarrays constructed from 305 breast cancer tissue samples. Next, stable knockdown of PHGDH in aggressive MDA-MB-231 breast cancer cells was carried out by shRNA-mediated silencing and the effect on cell proliferation analysed by the AlamarBlue Assay. A positive correlation was noted between PHGDH and PCNA in breast cancer tissues, whereby higher PHGDH immunostaining was associated with increased PCNA expression. Down-regulation of the PHGDH was observed to be concomitant with decreased growth of breast cancer cells in vitro, thereby, corroborating the finding in the clinical samples. PHGDH plays an essential role in proliferation, which is one of the hallmarks of cancer. PHGDH is, therefore, a potential biomarker for breast cancer cell proliferation and a molecular target for therapy.

Keywords: Phosphoglycerate dehydrogenase; Proliferating Cell Nuclear Antigen; Immunohistochemistry; shRNA-mediated silencing; Breast cancer cell proliferation
Effect of oleuropein on histopathological changes of mouse skin carcinogenesis model

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Abstract

Oleuropein is a phenylethanoid, a form of a phenolic compound which can be majorly found in olive leaves. Previous studies have shown several pharmacological activities of oleuropein against different types of cancer. In this study, we further examined the effect of oleuropein on the histopathological changes of the two-stage skin carcinogenesis mouse model. Total of 30 female ICR mice was randomly divided into 5 groups (n=6 per group) with 3 treatment groups and 2 control groups. Group I was pre-treated with oleuropein (10mg/kg) before DMBA/TPA application, group II was treated with oleuropein (10mg/kg) post-DMBA, which was prior to TPA, group III was treated with oleuropein (10mg/kg) at both pre-DMBA and pre-TPA applications, group IV was the carcinogen control treated with DMBA/TPA and group V as a control that received 70% acetone only. All animals were sacrificed at week 16. The skin and tumour tissue samples were biopsied for histopathological examination. At week 16, the treatment group I displayed mild epidermal hyperplasia with the basement membrane remained intact to the dermal area. On the other hand, the treatment group II and group III exhibited similar histological findings. Both groups showed a papilloma formation with hyperkeratosis. While carcinogen control group IV demonstrated a well-differentiated squamous cell carcinoma at week 16 with the presence of the mitotic body and keratin pearls, compared to control group V that remained normal. Thus, this may indicate the stage-specific role of oleuropein in inhibiting tumour development upon pre-treatment at the initiation stage as compared to pre-treatment at the promotion stage. Oleuropein has a potential chemopreventive act against the development of mouse skin carcinogenesis through its anti-carcinogenic efficacy on pre-cancerous cells, and it is suggested that additional studies be carried out to corroborate these findings.

Keywords: Oleuropein; Carcinogenesis; Skin cancer; Chemopreventive; Carcinoma

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Hibiscus sabdariffa polyphenol-rich extract preserves cardiac function and prevents cardiac injury in type-1 induced diabetic model

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Abstract

Hibiscus sabdariffa or roselle has been widely proven as an antioxidant and has potential in treating cardiovascular disease especially in diabetic condition. This study aimed to determine the protective effects of H.sabdariffa polyphenol-rich extract (HPE) in attenuating cardiac functional and structural abnormalities in type-1-induced diabetic rats. Male rats were divided into four groups: non-diabetic (NDM), diabetic without treatment (DM), diabetic supplemented with HPE (DM+HPE) and metformin (DM+MET). Type-1 diabetes was induced with streptozotocin (55 mg/kg/i.p). Rats were forced-fed with HPE (100 mg/kg) and metformin (150 mg/kg) daily for eight weeks after diabetic confirmation. Results showed that HPE supplementation improved hyperglycemia significantly (p<0.05) in DM+HPE compared to DM group. HPE supplementation attenuated cardiac oxidative damage in DM group, indicated by low malondialdehyde and advanced oxidation protein product. HPE significantly (p<0.05) increased reduced glutathione level, as well as catalase and superoxide dismutase 1 and 2 activities. These findings correlate with cardiac function, whereby HPE supplementation improved left developed ventricular pressure, coronary flow, cardiac contractility and relaxation rate significantly (p<0.05). Histological analysis showed a marked decrease in cardiomyocyte hypertrophy and fibrosis in DM+HPE compared to DM group. Immunohistochemistry stains for cleaved caspase-3 showed a marked increase in cardiomyocyte apoptosis in DM group and notably down-regulated by HPE supplementation. Ultrastructural changes and impairment of mitochondria induced by diabetes were minimized by HPE supplementation. These findings suggest that HPE was effective in attenuating cardiac functional and structural abnormalities in diabetic rats through by acting as a potent antioxidant against the products of oxidative stress.

Keywords: Diabetes mellitus; Hypertrophy; Apoptosis; Fibrosis; Hibiscus sabdariffa

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Monosodium glutamate daily oral supplementation based on average human intake: Study of its effects on sperm quality and testicular oxidative stress on rats model

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Abstract

Monosodium glutamate (MSG) is widely used in food preparation and industry and has regularly been consumed. Previous studies reported on the effects of MSG when given at extremely high dosages, which results are not applicable to the human equivalent intake. Therefore, the present study aimed to study the effect of MSG on sperm quality and reproductive organs changes of adult male rats when taken at average daily intake (ADI) estimated for a human. 24 adult male rats were randomly assigned into three groups; NC (Normal control), M60 and M120 where MSG was given orally at 60 mg/kg and 120 mg/kg to the respective group. All treatments were conducted for 28 consecutive days. Supplementation of MSG at estimated ADI (120 mg/kg body weight) causes a significant decrease in sperm quality (p<0.05) when compared to both control and M60 groups. Weights of reproductive organs were also significantly diminished (p<0.05). In addition, analysis of oxidative status showed that supplementation of MSG induces oxidative stress in the testis, even much more severely at dose 120 mg/kg body weight. These findings are supported by alterations in the histoarchitecture observed in the histology of reproductive organs. This finding revealed that MSG taken at 120 mg/kg body weight (ADI) daily could cause significant damage to the reproductive system.

Keywords: Food enhancer; Testis; Sperm quality; Monosodium glutamate; Accessory organs
Proceedings

Low dose effects of monosodium glutamate on biochemical and histopathological changes in the liver of male Sprague dawley rats

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Abstract

Monosodium glutamate (MSG) is widely used as an additive in daily food intake. Excess consumption of MSG was reported to cause oxidative stress on the brain, liver and renal resulted in the increased production of reactive oxygen species (ROS). This study aimed to determine the biochemical and histological effects of MSG on the liver functions level, status of oxidative damage, antioxidant levels and histological changes in the liver of adult male Sprague Dawley rats. The male Sprague Dawley rats (n=24) were randomly divided into three groups with two treatment groups (n=8 rats per group) and one control group (n=8). The treatment groups were administered MSG in a dose of 60mg/kg (MSG60) and 120mg/kg (MSG120) body weight respectively, and the control group received distilled water. The substances were administered to the rats via force feed for 28 consecutive days. The body weight, food and water intake of the rats were monitored every week. At day 29, all rats were sacrificed, and the liver was dissected out for biochemical and histological analysis. Liver enzyme level of aspartate aminotransferase (AST) together with total protein content demonstrated a significant increased (p<0.05) in MSG120 treatment groups compared to the control group. The antioxidant level of SOD in the liver tissue showed a significant increased (p<0.05) in MSG120 group compared to the control group. In contrast, the GSH levels in the liver tissue showed a significant reduction (p<0.05) in MSG120 group compared to the control group. For the status of oxidative damage, a significant increased (p<0.05) of MDA levels was displayed in MSG120 group compared to the control group. The histological findings revealed changes to liver architecture and haemorrhage in the central veins of the liver in MSG group rats. This study indicates that the low dose of MSG consumption particularly at 120mg/kg may have some damaging effects on the biochemical and histological parameters of the liver. Thus, the acceptable dose of MSG intake should be possibly reviewed, and additional studies to support these findings will be commenced in the future.

Keywords: Monosodium glutamate; Liver; Oxidative stress; Antioxidant; Histopathology
Microscopic evaluation of different hydroxyapatite scaffold from nacre layer for bone tissue engineering

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Abstract

Hydroxyapatite scaffold is preferred in bone regeneration due to its similar composition and structure with the natural human bone. It can be obtained from natural sources such as mollusc species. This study was conducted to compare between two different preparation of hydroxyapatite scaffolds from the nacre layer seeded with pre-osteoblast cells using scanning electron microscopy. MC3T3-E1 pre-osteoblast cells were seeded on two groups of spherical-shaped hydroxyapatite scaffolds from nacre layer (sinter and non-sinter) on 24-well culture plates. Media for cells cultured on both scaffolds were changed every three days and kept in incubators for 21 days. Observation using Field Emission Scanning Electron Microscopy (FESEM) was carried out on day 21 of in vitro incubation. Cellular proliferation and colonisation were evident on sinter hydroxyapatite scaffolds than in non-sinter hydroxyapatite by day 21. Furthermore, the sinter group has larger micropores as compared to the non-sinter group. Proliferation and colonisation of pre-osteoblast cells are more optimum in sinter HA scaffolds. This type of thermally sinter scaffolds is associated with larger micropores which may allow better cell adhesion and proliferation. Hence, it is suggested that this type of HA scaffolds could be used as useful scaffolds in bone tissue engineering.

Keywords: FESEM; Sinter hydroxyapatite scaffold; Bone engineering
Anti-tumour potential of bee venom and its component melittin in gastric cancer in vitro

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Abstract

Bee venom (BV) has been traditionally used in the treatment of numerous diseases such as arthritis, back pain and multiple sclerosis. Melittin is the significant component in BV isolated from honey bee Apis mellifera. Both BV and melittin have been previously reported to possess anti-tumour activity. In the present study, the cytotoxicity of BV and melittin were evaluated in gastric cancer in vitro. Gastric cancer cell lines (MKN-7, MKN-74 and NUGC-3) were routinely cultured. Functional studies such as Lactate dehydrogenase (LDH) cytotoxicity assay, cell cycle PI-Annexin V assay were performed to confirm the exerted cytotoxicity effect. Migration and invasion ability of gastric cancer cells were examined using Transwell and Matrigel chambers. Our results demonstrated that BV and melittin could inhibit the growth of gastric cancer cell lines, particularly the poorly differentiated NUGC3 gastric cancer cell line. In addition, BV and melittin also increased LDH release, with nuclear changes, the formation of apoptotic bodies and membrane blebbing, indicating that both BV and melittin could induce cell death mechanism through the apoptotic pathway. Cell cycle and PI-Annexin V analysis showed a significant percentage of cells in the sub-G1 phase which had undergone apoptosis. In addition, BV and melittin were also observed to attenuate the migratory and invasive potential of gastric cancer cells. The present data provide that BV and melittin could be potentially useful candidate compounds for further exploration as anti-tumour agents in gastric cancer.

Keywords: Bee venom; Melittin; Anti-tumour; Cytotoxic; Apoptosis
Low dose of monosodium glutamate-induced oxidative damage and histopathological changes on kidney of male *Sprague dawley* Rats

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Abstract

Monosodium glutamate (MSG) is a flavour enhancer which has been reported to cause oxidative damage in the male reproductive system, liver, red blood cells and bone marrow hence its usage is still controversial. However, up to date, there is no study done on the effects of a low dose of MSG on the oxidative stress status and histopathological observation of kidney. A total of 18 male *Sprague Dawley* rats, weight between 250-300g were divided randomly into three groups consists of control (received distilled water = 1 ml/kg), MSG 60 (received 60 mg/kg MSG) and MSG 120 (received 120 mg/kg MSG) groups. All the substances were given for 28 consecutive days. At the end of the study, all rats were sacrificed, and kidneys were isolated for histology and biochemical evaluation. Superoxide dismutase (SOD) activity and protein carbonyl (PC) level showed significantly increased (*p*<0.05) in MSG 120 group compared to the control group and MSG 60 group. However, no significant differences were found in Glutathione (GSH) and malondialdehyde (MDA) levels of all treated groups (*p*>0.05). Meanwhile, the histopathological observation showed vasodilation and glomerulus shrinkage in MSG 120 group. The repeated exposure of MSG, especially at the dose of 120 mg/kg can cause oxidative damage and histopathological changes on the kidney of male *Sprague-Dawley* rats.

*Keywords: Oxidative stress; Antioxidant; Reactive Oxygen Species; Protein oxidation; Lipid peroxidation*

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Roselle polyphenols prevents hyperglycemia-induced oxidative stress and mitochondria damage in rats heart

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Abstract

Hyperglycemia-induced myocardial and mitochondrial damage play essential roles in the initiation of cardiovascular diseases. Hibiscus sabdariffa or roselle has widely known for its antioxidant properties. This study aimed to investigate the ameliorations of hyperglycemia-induced oxidative stress and mitochondria damage by roselle polyphenol-rich extract in type-1 diabetic rats heart. Male Sprague-Dawley rats (200-250g) were used in this study. Rats were divided into four groups: Non-Diabetic (NDM), diabetic without treatment (DM), diabetic with roselle polyphenol (D+Roselle) and diabetic with Metformin (DM+Met). Type-1 diabetes was induced by a single dose of streptozotocin (55 mg/kg, intraperitoneal). Diabetic rats were orally fed with 100 mg/kg of roselle’s polyphenols or Metformin (DM+MET) at a dose of 150mg/kg for four weeks duration. Results showed that DM+Roselle could reduce blood glucose and lipid profile significantly (p<0.05) when compared to the DM group. DM group had significantly (p<0.05) higher level of malondialdehyde and advanced oxidation protein product than DM+Roselle group. Roselle treatment also significantly restored activities of superoxide dismutase 1 and 2, catalase as well as reduced glutathione levels. Observations using electron microscope showed that the appearance and the organisation of mitochondria in the NDM group were organised and more elongated compared to DM group. In conclusion, these results highlight the role of Roselle as a protective agent against myocardial injury and mitochondrial damage in diabetic rat model possibly by inhibiting oxidative stress.

Keywords: Roselle; Hyperglycemia; Free radicals; Myocardial damage; Mitochondria
Reusing archived formalin-fixed paraffin-embedded (FPPE) electromagnetic field (EMF) exposed blocks to resin

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Abstract

In our studies on low electromagnetic field (EMF) exposure, we observed that the tissues were affected at different damage spectrum. Some of the damages are not visible at the light microscopy level. Recently we have gone through our FPPE blocks (archived for 1-2 years) to tap more information ultra-histologically. The archived FPPE blocks used were remnants resections of tissues exposed to EMF at different intensity in vivo; control group, T1(0.5 mT) and T2(1.0 mT). The FPPE blocks were stored at temperatures maintained in the range of 17°C–22°C and humidity levels of 20%–60%. Briefly, post-fixation, the tissues were fixed in 10% phosphate-buffered formalin for 2h, followed by tissue processing for 10h, and paraffin embedding for 20 min. The area of interest was chosen and processed for electron microscopy staining. The staining control is with osmium tetroxide while the use of osmium tetroxide for treatment staining group is omitted. The study also performed staining with, and without the use of xylene during dewaxing, propylene oxide was replaced with acetone. There were alterations in the tissue structures exposed to the different intensity of EMF. The quality of the specimen image by osmium tetroxide while the use of osmium tetroxide for treatment staining group is omitted. The study also performed staining with, and without the use of xylene during dewaxing, propylene oxide was replaced with acetone. There were alterations in the tissue structures exposed to the different intensity of EMF. The quality of the specimen image by osmium tetroxide post-fixation showed the changes of tissue structures in the control group, T1 and T2 groups. The new protocols for upcycling the archived FPPE blocks to resin successfully retained the ultrastructural characteristics of the tissues exposed to EMF at different strengths. The apoptosis damage caused by the EMF was clearly shown.

Keywords: FPPE; EMF; Paraffin embedded; Resin embed; Electromagnetic

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Effects of pre-mineralization of nanobiocomposite bone scaffold in SBF towards bone marrow-derived stem cells (BM-SCs) growth and differentiation

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Abstract

Potential bone grafting materials such as scaffolds are widely being developed as part of the ever-expanding field of bone tissue engineering. In our previous study, we have successfully developed and characterized a three dimensional nanobiocomposite bone scaffold using biomaterials such as nano cockleshell powder and alginate. The nanobiocomposite scaffold (Alg/nCP) has been shown to possess ideal characteristics and excellent osteoconductive properties. Apatite layers formed by simulated body fluid (SBF) on surface of calcium-based scaffolds has been proven to enhance osteoblastic activity of pre-osteoblast and osteogenic activity of bone marrow derived stem cells (BM-SCs). In this study, we aim to determine the potential use of Alg/nCP bone scaffold and the effects of pre-mineralization of the scaffold in SBF in the growth and differentiation of BM-SCs. Scaffolds that were not pre-mineralized in SBF were used as controls for the study. SEM observations at 14 days of culture period revealed proliferation and growth of BM-SCs with presence of surface mineralization in both SBF treated and control scaffolds. Findings from this study, however, showed a significant decrease in cell proliferation as quantitated through MTT proliferation assay in SBF group during its culture period. Deposition of calcium on the scaffolds through Von Kossa histology staining showed presence of higher areas of calcium deposits in the control scaffolds compared to SBF scaffolds. ALP analysis as an early biomarker for BM-SCs osteogenic differentiation showed significant decrease in SBF group comparatively. In conclusion, Alg/nCP bone scaffolds did support BM-SCs’ growth however, pre-mineralization of scaffolds in SBF did not provide a better environment for the growth and proliferation of the cells.

Keywords: Nanobiocomposite scaffold; Simulated body fluid; Bone marrow-derived stem cell
Extremely low electromagnetic field (1.0 mT, 50 Hz) exposure affects osteoblasts growth on nanobiocomposite bone scaffold

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Abstract

Electromagnetic field is an effective application for bone regenerating activity by promoting osteoblast growth. However, the evidence of EL-EMF stimulation as therapeutic medicine is still limited. The purpose of this study was to observe bone tissue formation in vivo and cells growth when exposed to different EL-EMF intensities. Bone scaffold was developed from a mixture of 40% alginate and 60% nano-cockle shell powder. Bone scaffold was prepared in 10x3x5 mm³ cylindrical implant and seeded with 1.0 x 10⁶ osteoblast prior to subcutaneous implantation on the right and left dorsum of Wistar rats. A total of 18 male Wistar rats were randomly divided into three groups: control group (NC) that was not exposed to EL-EMF and treatment groups that were exposed to EL-EMF at 0.5 mT (T1) and 1.0 mT (T2) for an hour daily for the duration of two weeks. Hematoxylin & Eosin staining showed osteoblast infiltration and blood vessels formation were found to be higher in T2. Masson’s Trichrome staining showed the presence of collagen in NC, T1 and T2. Von Kossa staining revealed the accumulation of calcium in T2 was higher than NC and T1. ALP analysis was found significantly higher in T1 (p<0.05) as compared to NC and T2. Osteoblast counts revealed a significant increase (p<0.001) in T2 as compare to NC and T1. The findings from this study indicate the possible use of EL-EMF as an alternative therapy that could accelerate the process of bone tissue regeneration.

Keywords: Extremely Low Electromagnetic Field (EL-EMF); Bone scaffold; Osteoblast
Role of miR-26b and its target gene, Mecp2 in synaptogenesis in mouse embryonic neural stem cells from diabetic pregnancy

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Abstract

Maternal diabetes is known to cause neural tube defects (NTDs) in embryos and neuropsychological deficits in infants. Over the years, several metabolic pathways and a plethora of genes have been identified to be deregulated in developing brain of embryos by maternal diabetes, although the exact mechanism remains unknown. Recently, miRNAs have been shown to regulate brain development and maturation. Therefore, we hypothesized that maternal diabetes alters the expression of miRNAs that regulate genes involved in biological pathways critical for neural tube development and closure during embryogenesis. In order to address this, embryonic neural stem cells (NSCs) from normal and diabetic pregnancy were isolated from ICR mice and cultured in vitro. High throughput miRNA expression profiling revealed altered expression of several miRNAs in NSCs isolated from brains of streptozotocin-induced diabetic pregnancy when compared to control. Among the differentially expressed miRNAs, expression of miR-26b was significantly upregulated in NSCs from diabetic pregnancy when compared to control. Among the several predicted targets of miR-26b, methyl CpG binding protein2 (Mecp2) which is involved in brain development, synaptic maturation and plasticity was selected for further analysis. In addition, Mecp2 was significantly downregulated in NSCs from diabetic pregnancy when compared to normal. Further, loss/gain of function studies in NSCs revealed that Mecp2 is a direct target of miRNA-26b. Furthermore, knockdown of Mecp2 resulted in alterations in synaptic proteins - downregulation of Post synaptic density protein 95 (Psd-95) and upregulation of Synaptophysin and Clathrin 1 HC. Tau, a protein that stabilizes microtubules in axons of neurites in differentiated NSCs was also downregulated following Mecp2 knockdown. Taken together, this study reveals that maternal diabetes alters miR-26b expression resulting in down regulation of Mecp2 which in turn alters the expression of synaptic proteins. Thus, these alterations may lead to defective synaptogenesis that may manifest as neuropsychological disturbances in offspring of diabetic mothers.

Keywords: miR-26b; Mecp2; Synaptogenesis; Embryonic neural stem cells
The effect of gonadotropin releasing hormone on the expression of luteinizing hormone and estrogen in the ganglia and ovary of the tropical abalone *Haliotis asinina*

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Abstract

Gonadotropin releasing hormone (GnRH) is a peptide hormone which plays a role in the control of the reproductive function in both vertebrates and invertebrates. In vertebrates, GnRH is synthesized in the hypothalamus and transported to the pituitary gland where it stimulates secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from pituitary gland, which in turn stimulates gonadal development. However, the knowledge of GnRH and other reproductive hormones in the abalone is inadequate. In this study, we aim to investigate the effect of GnRH on the expression of LH and estrogen in the ganglia and ovary of *Haliotis asinina* by ELISA and immunohistochemistry techniques. Groups of one-year-old female abalone at mature phase were injected with the synthetic *H. asinina* (Has) GnRH at doses of 250 ng/gBW stimulated LH and estrogen release in the ganglia and the ovary, respectively (*P*<0.05). However, the HasGnRH at high dose of 500 ng/gBW had a lesser effect on inducing LH and estrogen release. For the immunohistochemistry, LH-immunoreactive cells were slightly observed in the cerebral and pleuropedal ganglia. In addition, LH-immunoreactive nerve fiber bundles were strongly detected in both ganglia. In the ovary, the immunoreactivity against estrogen appeared to be localized in the cell within the connective tissue and trabeculae. There was no positive staining in the cytoplasm of any stage of germ cells. These results provide important knowledge on the reproductive hormones which could be applied in abalone aquaculture to stimulate the ovarian maturation.

Keywords: Gonadotropin releasing hormone; Luteinizing hormone; Estrogen; Haliotis asinina