2010 GCOE Graduate Student Minisymposium

Date: Friday July 30

Start: 10:30 am

End: 12:00 pm (noon)

Place: Seminar Room on the first floor, Basic Research Building, Tsurumai Campus

Speakers & Titles:

Keisuke Kuroda
Reevaluation of DISC1 using knockout mice and new antibodies

Qiang Qiang
17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration

Mohammad Nizam uddin
GADD34 induces autophagy through the suppression of the mTOR pathway during starvation

Habibul Bari Shozib.
Choline acetyltransferase mutations cause myasthenic syndrome (CMS-EA) associated with episodic apnea in humans

Hirotaka Nagai
Diameter of multi-walled carbon nanotubes and nanofibres is a critical factor in mesothelial injury and subsequent inflammation

Mohammad Alinoor Rahman
Molecular pathomechanism of aberrant splicing of exon P3A in CHRNA1 due to a mutation causes congenital myasthenic syndrome

Each presentation will last 10 minutes followed by 2 minutes for questions.
Keisuke Kuroda

Year in PhD Program: 4th
Nationality: Japan
Graduated From: Nagoya University School of Medicine
Mentor: Kozo Kaibuchi (Pharmacology)

Title: Reevaluation of DISC1 using knockout mice and new antibodies

Background: Translocation of the Disrupted in Schizophrenia 1 (DISC1) gene is associated with schizophrenia. This gene was originally identified in the large Scottish family in which a balanced t(1;11) translocation that co-segregates with schizophrenia and other major mental illnesses. This translocation disrupts the DISC1 locus, and may result in loss of function via haplo-insufficiency or dominant-negative effects of a predicted truncated protein product. Recent studies have reported many functions and many interacting proteins of DISC1. According to these studies, DISC1 has multiple splice variants, expresses in both neuron and glia, and plays multiple roles in neuronal development through its interactions with different partners.

Problem: in vivo functions of DISC1 have not been defined, particularly in knockout mice.

Hypothesis: DISC1 is essential for normal embryonic development, and loss of DISC1 will produce abnormal, schizophrenic mice.

Methods: DISC1 knockout mice, and novel DISC1 antibodies, were generated to evaluate the effects of DISC1 in vivo.

Results: Unexpectedly, DISC1 homozygous deletion mice are viable and fertile, and no gross phenotype is observed. Using our novel antibodies, we also found that DISC1 does not have splicing variants, does not express in some mice strains, and is detected in many tissues during development, but hardly detectable in adult mice. We are now performing immunohistochemical and behavior analysis to further evaluate reported DISC1 functions.

Conclusions: DISC1 may not be required for normal mouse embryogenesis or behavior.

Novelty: The biological role of DISC1 and commercial antibodies used to evaluate its expression may need to be reevaluated.


Participants: Uddin & Shozib
Title: 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration.

Background: Heat-shock protein 90 (Hsp90) functions as a multichaperone complex that folds, assembles and regulates homeostasis of its client proteins. Androgen receptor (AR), a pathogenic gene product in spinal and bulbar muscular atrophy (SBMA), is an Hsp90 client protein. Previous studies demonstrate that Hsp90 blockers can induce AR degradation in prostate cancer cells.

Problem: Currently, there are no effective treatments for SBMA.

Hypothesis: 17-allylamino-17-demethoxygeldanamycin (17-AAG), a potent Hsp90 inhibitor, can degrade mutant AR proteins via the ubiquitin-proteasome system to ameliorate SBMA.

Strategy: Study the therapeutic effects of 17-AAG in cells and in a transgenic mouse model of SBMA.

Results: In cell culture, 17-AAG reduces AR aggregation and increases AR clearance via the ubiquitin-proteasome system. In vivo, SBMA transgenic mice treated with 17-AAG shows evidence of decreased mutant AR compared to control mice. Administration of 17-AAG also rescues behavioral and histopathological abnormalities, and reduces both muscle and spinal cord pathology, of SBMA mice.

Conclusions: 17-AAG, an Hsp90 inhibitor, can degrade mutant AR to ameliorate SBMA.

Novelty: Hsp90 inhibitors, such as 17-AAG, can degrade mutant AR and provide new and effective treatments for SBMA.


Participants: Uddin & Shozib
Mohammad Nizam uddin

Year in PhD program: 4th
Nationality: Bangladesh
Graduated from: University of Dhaka, Bangladesh.
Mentor: Prof Kenichi Isobe (Immunology)

Title: GADD34 induces autophagy through the suppression of the mTOR pathway during starvation.

Background/Significance: Several types of cellular stress including DNA damage, heat shock, nutrient deprivation, energy depletion, and ER stress induce expression of growth arrest and DNA damage protein 34 (GADD34). Autophagy has survival-oriented functions, occurring under both basal conditions and conditions of stress, such as starvation.

Problem: GADD34 and autophagy are both induced under starvation conditions. However a role for GADD34 in starvation induced autophagy has not been defined.

Hypothesis: Starvation induces GADD34 expression to promote autophagy through suppression of the mTOR pathway.

Methods: The effects of starvation on Gadd34 expression, mTOR activity, and autophagy were examined in wild type and homozygous null Gadd34 knockout mice.

Results: Starvation induced the expression of GADD34, reduced mTOR activity, and induced autophagy in wild type mice, but not Gadd34 KO mice. Gadd34 bound to and dephosphorylated pTSC2, an mTOR, activator, at Thr1462. Dephosphorylation of TSC2 during the starvation time period lead to the suppression of mTOR, which is a potent inhibitor of autophagy. In contrast, there was no difference in the expression pattern of pAMK or pERK in wild type and GADD34 knockout mice during the starvation time period.

Conclusions: Starvation induces Gadd34 suppresses mTOR and, thereby, induces autophagy.

Novelty: Gadd34 can function as a suppressor of mTOR and inducer of autophagy.


Participants: Nagai & Rahman
Habibul Bari Shozib.

Year in PhD program: 1st
Nationality: Bangladesh
Graduated From: University of Dhaka, Bangladesh
Mentor: Kinji Ohno (Neurogenetics & Bioinformatics)

Title: Choline acetyltransferase mutations cause myasthenic syndrome (CMS-EA) associated with episodic apnea in humans.

Background/Significance: Choline acetyltransferase catalyzes the reversible synthesis of acetylcholine (ACh) from acetyl CoA and choline at cholinergic synapses. This activity is suspected in causing CMS-EA in humans. Mutations in genes encoding ChAT affecting motility exist in Caenorhabditis elegans and Drosophila.

Problem: ChAT mutations have not been observed in humans, and its relationship to CMS-EA has not been defined.

Hypothesis: ChAT activity prevents congenital myasthenic syndrome associated with frequently fatal episodes of apnea (CMS-EA).

Methods: The ChAT gene was sequenced in number of people with CMS-EA. Mutant genes were transfected into cells in culture.

Results: 10 mutations were found in the ChAT gene in CMS-EA patients. A frameshifting null mutation (523insCC) and three point mutations (I305T, R420C, and E441K) mutations markedly reduce ChAT expression in COS cells. In addition kinetic studies of nine bacterially expressed ChAT mutants demonstrate that one mutant (E441K) lacks catalytic activity, and eight mutants (L210P, P211A, I305T, R420C, R482G, S498L, V506L, and R560H) have significantly impaired catalytic efficiencies of ChAT.

Conclusions/Answers: ChAT mutations are associated with, and may cause, CMS-EA in humans.

Novelty/Innovations: These findings represent the first time that ChAT mutations have been found in humans, and suggest a role for ChAT in CMS-EA.


Participants: Nagai & Rahman
Title: Diameter of multi-walled carbon nanotubes and nanofibres is a critical factor in mesothelial injury and subsequent inflammation

Background/Significance: Multi-walled carbon nanotubes (MWCNTs) have potential applications in various fields. However, due to their needle-like shape and high durability, concerns have been raised that MWCNTs may induce mesothelioma.

Problem: Previous studies demonstrate the potential cytotoxicity of MWCNTs, but the features of MWCNTs that determine mesothelial cell cytotoxicity remain unclear.

Hypothesis: The diameter of multi-walled carbon nanotubes and nanofibres is a critical factor in mesothelial injury and subsequent inflammation

Methods/Strategy: We added fibers to mesothelial cells and macrophages and observed fiber internalization into the cells, and their effects on cell viability. We also injected fibers into the peritoneal cavity of rats.

Results: Here we show that deleterious effects of MWCNTs on human mesothelial cells stem not from active phagocytosis, but from passive penetration. Thin dispersed MWCNTs with high crystallinity ($\varphi \sim 50$ nm) showed mesothelial cell penetration and cytotoxicity in vitro and induced severe fibrotic inflammation in vivo, whereas thick ($\varphi \sim 150$ nm) or aggregative MWCNTs ($\varphi \sim 2-20$ nm) did not. Notwithstanding this, every MWCNT studied contributed to macrophage-dependent local granuloma formation.

Conclusions/Answers: Thus, the diameter of MWCNTs is a critical factor for cytotoxicity in mesothelial cells and subsequent diffuse inflammation.

Novelty/Innovations: This work suggests that modulating the diameter of MWCNTs in production could reduce the threats to human health.


Participants: Kuroda & Qiang
Mohammad Alinoor Rahman

Year in PhD Program: 1st  
Nationality: Bangladeshi  
Graduated From: University of Dhaka, Bangladesh  
Mentor: Kinji Ohno (Neurogenetics)

Title: Molecular pathomechanism of aberrant splicing of exon P3A in CHRNA1 due to a mutation causes congenital myasthenic syndrome.

Background: Humans and great apes have a 75-nt exon P3A in CHRNA1 encoding the muscle nicotinic acetylcholine receptor α-subunit. Alternative splicing generates two variants of the α-subunit, one with exon P3A and the other without P3A. The P3A(+) transcript encodes a nonfunctional α- subunit that is not expressed on the cell surface.

Problem: In normal muscle, the P3A(+) and P3A(-) transcripts are generated in a 1:1 ratio, but the functional significance and regulation underlying the alternative splicing remains elusive.

Hypothesis: The development of congenital myasthenic syndrome (CMS) involves splicing trans-factor(s) that regulate aberrant splicing of the P3A exon of the CHRNA1 gene.

Methods: We have sequenced all genes encoding subunits of the acetyl choline receptor (AChR) in a patient with CMS. We have also investigated the function of RNA splicing proteins involved in the regulation of these gene products.

Results: A mutation at the 23rd nucleotide of exon P3A causes exclusive inclusion of this exon in a patient with congenital myasthenic syndrome. This mutation changes the binding of RNA splicing regulatory proteins, from hnRNP L, which silences expression, to hnRNP LL, which enhances expression of exon P3A.

Conclusion: Recognition of exon P3A is physiologically suppressed by hnRNP L to make a functional P3A(-) transcript. A mutation in exon P3A of CHRNA1 disrupts binding of hnRNP L and enables binding of hnRNP LL, which produces a non-functional P3A(+) transcript, and leads to congenital myasthenic syndrome.

Novelty: Mutations in the CHRNA1 can cause CMS, and may present a new target gene for hnRNPs L and LL along with their novel mode of regulation.


Participants: Kuroda & Qiang