Winter 2012

ACTH Promotes Osteogenesis of Rat Mesenchymal Stem Cells through the Melanocortin-2 Receptor

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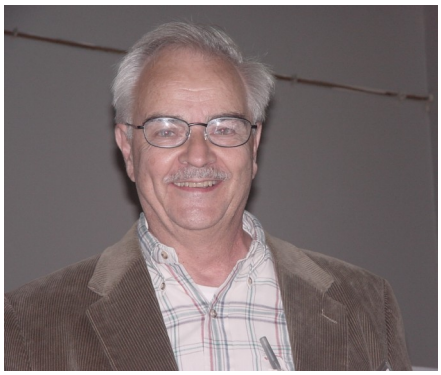
IN VIVO

The Publication of the Metropolitan Association of College and University Biologists

Winter 2012 Volume 33, Issue 2

44th Annual MACUB Conference
Seton Hall University
South Orange, New Jersey
October 29, 2011







Instructions for Authors

IN VIVO is published three times yearly during the Fall, Winter, and Spring. Original research articles in the field of biology in addition to original articles of general interest to faculty and students may be submitted to the editor to be considered for publication. Manuscripts can be in the form of a) full length manuscripts, b) mini-reviews or c) short communications of particularly significant and timely information. Manuscripts will be evaluated by two reviewers.

Articles can be submitted electronically to *invivo@mec.cuny.edu* or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - [1]Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32:** 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

The Effects of Exogenous Carbohydrates on Notch Binding to Its Ligand Delta. Stacy-Ann Robinson, Alimra Mohammed and Dan Moloney Queensborough Community College, Bayside, NY.

Notch signaling is vital to many cell processes including cell-cell communication and cell fate decisions. Notch malfunction has been implicated in such diseases such as Leukemia, Alagille syndrome and CADASIL. Glycosylation of Notch receptor impacts signaling. The carbohydrates on the exterior domain of Notch are an integral part of Notch's functionality as changes to the O-linked carbohydrates have been shown to affect Notch signaling. The enzyme Fringe is a glycosyltransferase that modifies O-linked fucose on the extracellular domain of Notch and alters receptor signaling. In this experiment we tested the impact of exogenously added sugars including fucose on Notch binding to its ligand Delta using cell based ligand binding assays. In the ligand binding assay, Delta tagged with heavy chain (Fc) from human immunoglobulin was applied to cells expressing Notch, and binding was measured using an anti-human secondary antibody conjugated with Horseradish peroxidase. The results of our assay showed that addition of exogenous fucose reduced Delta binding to Notch, whereas addition of exogenous para-Nitrophenyl fucose surprisingly increased Delta binding to Notch. Discovering compounds that impact ligand binding to Notch will help researchers to manipulate cell differentiation pathways and better understand Notch signaling.

ACTH Promotes Osteogenesis of Rat Mesenchymal Stem Cells through the Melanocortin-2 Receptor. Sylvana Rodriguez[1,2], Jodi Evans[1,2] and Louis Ragolia[2]. [1]Molloy College, Rockville Centre, NY and [2]Winthrop University Hospital, Mineola, NY.

Adrenocorticotropin Hormone (ACTH) is an endocrine hormone that is secreted by the pituitary and stimulates the secretion of cortisol from the adrenal cortex. It is among the several melanocortin peptide hormones that are derived from proopiomelanocortin (POMC) such as α-melanocyte stimulating hormone (α-MSH), γ-MSH and the endorphins. ACTH is also produced by cells outside the central nervous system and has been found to play a role in osteogenesis. Using mesenchymal stem cells (MSC) obtained from bone marrow of the Wystar Kyoto (WKY) rat, we confirmed that ACTH increases osteogenesis in a dose-dependent manner. Immunoblot of crude membrane fractions was used to determine that rat MSC express three melanocortin receptors (MC-R); the MC2-R, MC3-R and MC5-R. To determine which of these receptors mediate ACTH-induced osteogenesis we used MC-R specific peptides and antagonists. Neither α-MSH, a strong agonist of the MC5-R nor γ_2-MSH, a strong agonist of the MC3-R, increases osteogenesis in rat MSC. Additionally the MC3-R specific antagonist did not suppress ACTH-induced increases in osteogenesis. In addition, calcium flux was examined as a mechanism for ACTH action at the MC2-R. Consistent with MC2-R expression patterns in the MSC cultures, ACTH-induced transient increases in intracellular calcium were increased with dexamethasone treatment. Therefore the osteogenic effects of ACTH in rat MSC cultures are consistent with an MC2-R signaling mechanism. This pathway represents a new therapeutic target in the prevention and treatment of bone loss.

***Haplosporidium nelsoni* (MSX) Small Subunit rRNA Gene is Detected in the Eastern Oyster (*Crassostrea virginica*) in Jamaica Bay, NY. Keisha Rogers, Craig Hinkley, Gary Sarinsky. Kingsborough Community College, Brooklyn, NY.**

Haplosporidium nelsoni (MSX) is a parasitic protozoan which is responsible for extensive mortality in the Eastern Oyster (*Crassostrea virginica*) populations along the eastern coast of North America. The origin of MSX to these waters is unknown. However, it is speculated that it was introduced when the Pacific Oyster (*Crassostrea gigas*) was attempted to be used as a replacement for the declining native oyster stocks in the Delaware and Chesapeake Bays during the late 1950`s. As of now, the intermediate host or hosts that transmit MSX to oysters are unknown. There has been no known oyster populations observed in Jamaica Bay since the 1920`s. Oysters used in this experiment were grown from spats in Taylor Floats in the Bay. It is hypothesized that the unknown host or hosts would be present in Jamaica Bay and there will be oysters found infected with MSX. DNA was isolated from gill and mantle tissues. PCR amplifications were carried out using the MSX small subunit rRNA and the mitochondrial cytochrome c oxidase 1 (CO1) gene primer sets respectively. The MSX amplified products and a positive control for MSX were then subjected to agarose gel electrophoresis to determine correct size (564-bp). One of the six oyster`s gill and mantle tissue and the control were positive for MSX. To verify that DNA was obtained from all of the oysters, the CO1 amplified products were subjected to agarose gel electrophoresis to determine correct size (702 bp)and was found to be present in all six samples. The MSX amplified and CO1 amplified products were sequenced and were subjected to NCBI blast searches which further verified that the small subunit rRNA gene was from *Haplosporidium nelsoni* and the CO1 gene from *Crassostrea virginica*. The results of these experiments demonstrate that MSX is present in *Crassostrea virginica* in Jamaica Bay.

Polyketide Synthase is Found in Lichen Species in Metropolitan New York and Sub-Sahara Africa Areas. Mojisola Rotibi and Ivan Shun Ho. Kingsborough Community College, Brooklyn, NY.

Lichens are symbiotic organisms of fungi, algae, and cyanobacteria. There are approximately 13500 Lichen species worldwide and more than 1200 can be found in South Africa. Lichens can synthesize numerous secondary metabolites, known as the "lichen acids". To date over 1000 lichen secondary metabolites have been identified. Just like the amount of lichen species, these metabolites have diverse functions: antiviral, antibiotic, antitumor, etc. Some of them even allow lichens to thrive in locales that are deemed uninhabitable. An essential step in the production of secondary metabolites is conducted by an enzyme called polyketide synthase (PKS). We investigate whether the conserved ketosynthase (KS) domain of PKS is present in lichens from areas of New York and South Africa. We hypothesize that samples from New York will possess PKS for the poor air quality of lower Manhattan. In addition, we hypothesize that the more populated Cape Town has considerable air pollution and therefore lichens of that region will have PKS, whereas lichens found in the coastal, less populated Mozambique Archipelago will not. Lichen DNA was isolated using a protocol involving Edward's Buffer. The KS domain of PKS was targeted and amplified by polymerase chain reaction (PCR) using degenerate primers. A region of tubulin was also amplified as a control. The PCR products were separated by agarose gel electrophoresis to verify the correct size. Our preliminary data show that the lichen species collected in Cape Town does have the KS domain of the PKS in their genome while species from Mozambique do not. Interestingly, our data also show that samples from Battery Park City possess the KS domain while those from Brooklyn Heights do not. This work was supported by Grant 2R25GM06003-05 of the Bridges to the Baccalaureate Program of NIGMS and Grant 0537101091 of the CSTEP Program of the New York State Department of Education.