CAANCB Student Oral Presentation Abstracts

C01

PROEPIDERMAL GROWTH FACTOR CYTOPLASMATIC DOMAIN AFFECTS GROWTH OF HUMAN THYROID CARCINOMA BY A PROCESS INVOLVING PROTEASOMAL DEGRADATION.

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We have studied the role of the cytoplasmic domain of human EGF (proEGFcyt) and a novel splice version lacking exon 23 (proEGFdel23) in stable transfectants of the human thyroid carcinoma cell line FTC-133. We provide first evidence that over-expression of human proEGFcyt, but not proEGFdel23 or mock controls, inhibits growth of human thyroid carcinoma cells. Exposure of these stable transfectants to exogenous EGF antagonizes this growth-suppressive action of proEGFcyt and causes a marked increase in tumor cell proliferation by EGF-mediated binding to and activation of EGF receptor (EGFR) as determined by the specific EGFR inhibitor AG1478. ProEGFcyt transfectants display significantly reduced presence of EGFR but the amount of EGFR protein sharply increases in the presence of two proteasome inhibitors, MG132 and Lactacistin. How does proEGFcyt enhance proteasomal degradation of EGFR? We demonstrate that overexpression of proEGFcyt causes a significant down-regulation of Ubiquitin C-terminal Hydrolase-L1 (UCH-L1) which is important for protein de-ubiquitinylation. This coincides with the higher level of ubiquitinylation of proteins in proEGFcyt transfectants. Thus, our investigations describe a novel proEGFcyt-mediated molecular mechanism affecting proteasomal degradation of the EGFR and this may impact on the growth characteristics of human thyroid carcinoma cells.

C02

METHODS IN STUDYING COLLAGEN: THE NEW AND REVISITING THE OLD.

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Collagen is a ubiquitous, structural, filamentous protein in the extracellular matrix (ECM). It is the most abundant protein in the body. Currently there are very few methods employed to quantify collagen. Literature review indicates some methods are ineffective while others are used incorrectly. Collagen is a large protein (190kDa), with many intermolecular cross-links; therefore the proteins do not run through a gel, but get stuck at the top of the wells. In an attempt to quantify collagen content using gel electrophoresis, we developed a new method utilizing a collagenase digestion step. Protein samples from skeletal muscle were digested with collagenase and aliquots were run on an 8% SDS reducing gel. After blotting and immune probing, bands were present at the 190kDa weight. Several other smaller bands were visible; these may be fragments as there were no bands present when collagen was not digested. This method detected tissue-specific differences in collagen content. With so few methods available, new methods are useful. However, it is important that we use the current methods appropriately. Sirius red F3B is a collagen-specific stain that stains several items red under bright field microscopy, including central nuclei in regenerating muscle. In contrast, when polarizing filters are used, only collagen is visible, because the birefringence is highly selective to collagen and is not observed in central nuclei. Certain dyes, such as Sirius red enhance our ability to observe the polarizing effect of collagen. Therefore, polarizing filters must be used when Sirius red is being used as a collagen specific stain, and other methods of studying Sirius red staining (e.g., by epifluorescence microscopy) will be non-specific. As collagen is one of the most critical components of the body and an important player in fibrotic diseases and inflammation, new ways of quantifying collagen and appropriate use of current methods as they were intended, are necessary for a rigorous approach to hypothesis testing.

C03

CHANGES IN STRUCTURE, STEM CELL ACTIVATION AND GENE EXPRESSION IN SKELETAL MUSCLE WITH AGE.

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The huge impact of age-related sarcopenia led to this research to re-examine the effects of age on factors that maintain muscle growth, namely fiber growth, precursor cell proliferation, and gene expression. Several variables were obtained from studies of normal C57BL6 mice at 3, 6, 8 and 12 months of age. Grip strength was measured using a Chatillon strain gauge. Immediately after euthanasia, extensor digitorum longus (EDL) and gastrocnemius (GAST) muscles were isolated and prepared for whole muscle culture. Tissues were assayed for DNA synthesis and fiber CSA, and protein and mRNA extracts were used to study gene expression. Rates of DNA synthesis (dpm/ug DNA) were obtained by culturing EDLs in basal medium and 3H-thymidine for 22 hours. Fiber CSA was acquired from 5 um sections of GAST using NIH ImageJ software. The number and distribution of satellite cells was determine using in situ hybridization for pax7, MyoD and myogenin mRNA. Levels of NOS-I, myostatin and myf 5 were obtained (Westerns) to mark regulators of growth (myostatin) and effects of age on stem cell activation (myf5). Data were compared using 2-way ANOVAs and Least Significant Difference statistical tests ($P<0.05$). Results indicated body mass (g), muscle mass (mg) and fiber CSA (um2) rose significantly up to 6 mo of age and then declined. Body mass was also significantly greater at 8 mo compared to 3 and 12 mo. By contrast, grip strength (g x 10/g BW) was greatest at 3 mo. Proliferation accounted for over 30% of the variation in muscle mass in a significant correlation. Preliminary in situ hybridization data suggest satellite cell pool size declines with age. Further experiments on changes in skeletal muscle with age are in progress to determine if these alterations are even more prevalent at 18 mo.

C04

THE INFLUENCE OF NICOTINE ON DMSO-INDUCED HUMAN LEUKEMIC PROMYELOCYTES AND SUBSEQUENT EFFECTOR FUNCTION.

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Neutrophils leave the bone marrow as terminally differentiated cells, yet little is known of the influence of tobacco smoke on neutrophil differentiation and subsequent effector function. In this study, the effects of nicotine (10-7M to 10-4M), a specific component of tobacco smoke, on DMSO-induced neutrophilc differentiation of HL-60 cells were examined by assessing (a) cell growth kinetics, (b) cellular morphology and ultrastructure, (c) the expression of CD11b, (d) the ability to mount an oxidative burst, and (e) growth phase and apoptosis. The expression of nicotinic acetylcholine receptors (nAChRs) in HL-60 cells was determined using western blot and immunofluorescence. The influence of nicotine on effector functions of DMSO-stimulated HL-60 cells were also studied, i.e. respiratory burst and matrix metalloproteinase (MMP) secretion. The results showed that both promyelocytic and neutrophil-differentiated HL-60 cells expressed the á7-nAChR subunit (55kDa). Expression of the á7-nAChR was up-regulated upon the addition of DMSO, irrespective of nicotine exposure. Nicotine exposure during differentiation suppressed the oxidative burst in HL-60 cells; inhibited bacterial killing; and increased the release of MMP-9, but not MMP-2, in an á7-nAChR-dependent manner. Nicotine did not significantly elevate the percentage of DMSO-undifferentiated or -differentiated HL-60 cells committed to apoptosis. Nicotine increased the percentage of cells in late differentiation phases (metamyelocytes, band cells and segmented neutrophils) compared to DMSO alone ($p < 0.05$). Furthermore, There were no statistically significant differences in any other marker of neutrophil differentiation between treated and control HL-60 cells (all $p > 0.05$). Thus, pharmacological doses of nicotine do not affect gross markers of neutrophil differentiation or induce commitment to apoptosis in the biochemical profiles of á7-nAChR-expressing HL-60 cells. Exposure to nicotine during cellular differentiation alters effector function in neutrophils which may partially explain the increase in susceptibility of tobacco smokers to bacterial infection.

C05

GLUTAMINE: A NOVEL THERAPEUTIC FOR ACUTE SPINAL CORD INJURY.

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Secondary damage after spinal cord injury is mediated by oxidative stress and inflammation. Following injury, concentrations of the potent antioxidant glutathione (GSH) are decreased in the spinal cord which potentiates mechanisms of secondary damage. In an attempt to maintain the GSH concentrations, the non-essential amino acid L-glutamine (GLN) was tested as it was shown to increase GSH concentrations both in vivo and in vitro. To examine the therapeutic potential of glutamine after spinal cord trauma, a compression injury was induced in male Wistar rats a modified forceps model. GLN was administered by intraperitoneal injection 1 hour after injury and twice daily for one week. Blood GSH measurements were taken pre-surgery, at time zero (1 hour post-injury and at the time of GLN administration) and 30 minutes, 1, 6, 24, 48 and 72 hours after. Weekly behavioral testing including inclined

plane and BBB testing evaluated functional recovery. Six weeks post-surgery animals were sacrificed and tissues were collected for histology including cresyl violet and luxol fast blue staining. An injury severity curve was generated to determine the effect of 1mmol/Kg GLN treatment following spinal compression to widths of 0.2, 0.4, 0.6, 0.8 and 1.0mm at spinal level T6. GLN supplementation was found to be most effective in moderately severe injuries (0.4mm and 0.6mm). In all severities GLN treatments increased blood GSH content at various time points, most consistently 24 hours post-injury. Using the 0.4mm injury a dose-response was generated determine the potency of GLN (0.5, 1.0, 2.5 and 5mmol/Kg), alanine (1mmol/Kg) following injury. The 1mmol/Kg concentration was found to be the most effective in increasing both BBB and inclined plane scores while alanine did not affect any measured outcome. One mmol/Kg GLN treatment also increased the amount of tissue spared 6 weeks following injury. These results indicate that GLN is an effective treatment for spinal cord injury as it significantly increases both functional recovery and amount of tissue spared following spinal cord injury.