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**Activin C: a possible therapeutic target in prostate cancer progression. E Ottley,
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Prostate cancer (PCa) is a worldwide health concern. Broadly, two forms of PCa exist, latent organ-confined and aggressive metastatic. Treatment options for latent PCa are available and effective, whereas treatment options are limited for metastatic PCa. Activin A is a negative growth regulator in the prostate thereby inhibiting PCa progression. Activin A bioactivity is normally tightly regulated via antagonists; activin C is an antagonist of activin A and over-expression is associated with prostate hyperplasia. Thus we proposed that increased activin C maybe associated with PCa progression.

To address our hypothesis, we assessed proliferating cell nuclear antigen (PCNA) as a marker of proliferating cells, Smad-2 (an activin A signalling molecule) and p53 in the prostate of transgenic mice (TG) aged 9 months over-expressing activin C compared with wild-type (WT) controls (WT = 4; TG = 6). Significant increases were evident for PCNA (mean \pm SEM, WT, 32.4% \pm 4.8 vs. TG, 42.9% \pm 2.2, $P < 0.05$, unpaired *t*-test) and p53 (WT, 27.6% \pm 0.43 vs. TG, 38.5% \pm 3.4, $P < 0.05$), while Smad-2 decreased (WT, 48.53% \pm 2.29 vs. TG, 25.17% \pm 1.39, $P = 0.001$). Low-grade prostatic intraepithelial neoplasia lesions, a precursor to PCa, were found in 30% of the TG mice.

Human prostate sections were assessed for activin staining intensity (where 0 = nil staining and 3.5 = intense staining), and Smad-2 signalling. Activin C was increased in PCa compared to benign prostatic hyperplasia (BPH) (PCa 3.1 \pm 0.3, BPH 1.8 \pm 0.4, $P < 0.001$) and Smad-2 positive nuclei decreased (27% \pm 0.6 vs. 16% \pm 1.3, $P < 0.001$).

Decreased Smad-2 in association with increased activin C indicates antagonism of activin A in the development of mouse and human prostate pathology, therefore activin C may be a novel therapeutic target to modulate PCa progression.

Identification of friend and foe: metagenomics of the oral cavity in health and disease D Sundaresan¹, M Cullinan¹, B Drummond¹, G Seymour¹, J Stanton², N Heng¹. ¹Sir John Walsh Research Institute, Faculty of Dentistry, ²Department of Anatomy & Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.

The human mouth is home to more than 700 microbial species, collectively known as the 'oral microbiota'. Many species, mainly bacterial, cause oral infections such as dental caries and periodontitis (gum disease). However, only a small fraction of oral inhabitants are culturable. This project aimed to characterise, using next-generation

DNA sequencing technology: (a) the oral bacterial diversity in human participants at different stages of life, and (b) identify any shifts in the bacterial population in individuals with caries or periodontitis.

Samples from five intraoral sites were taken from 18 children and 15 adults, including individuals who were either periodontally healthy or those that had caries or periodontitis. Genomic DNA was purified and then subjected to polymerase chain reactions targeting hypervariable regions of the bacterial 16S ribosomal RNA gene. High-throughput DNA sequencing utilised the GS-FLX Titanium System and the data were processed by the Ribosomal Database Project Pyrosequencing Pipeline. A list of the ten most abundant species, i.e., the “Top 10 List”, was then compiled for each oral sample.

Analysis of the Top 10 Lists revealed that there was a significant species shift from healthy to diseased states, i.e., from a mixture of Gram-positive (e.g., *Streptococcus*) and Gram-negative genera to a predominantly Gram-negative population. Surprisingly, bacterial pathogens such as *Streptococcus mutans* and *Porphyromonas gingivalis* were conspicuously absent in individuals with caries and periodontitis, respectively. Whereas *Prevotella denticola* was commonly encountered in periodontitis samples, the picture was less clear with dental caries in that there appeared to be a reduction in *Leptotrichia* species (a Gram-negative genus) with a concomitant increase in streptococci.

This project demonstrates that next-generation sequencing technology is a powerful tool to study the oral microbiota. Furthermore, results show that some of the bacterial culprits in caries and periodontitis may have less of a pathogenic role than previously believed.

Patterns of α -MSH-induced neuronal activation in the pregnant rat brain. E Scherf, S Ladyman, D Grattan. Centre for Neuroendocrinology and Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.

During pregnancy, food intake is increased despite an increase in plasma leptin levels, which would be expected to suppress food intake. At least part of leptin action is mediated by α -melanocyte stimulating hormone (α -MSH), a peptide released by pro-opiomelanocortin neurons. In response to elevated leptin, α -MSH is released, activating melanocortin receptors on target neurons in the paraventricular, ventromedial and arcuate nuclei of the hypothalamus to mediate leptin's anorectic effect. Therefore, it was hypothesised that leptin resistance during pregnancy may be associated with a loss of response to α -MSH in distinct hypothalamic regions. To test this hypothesis, we used immunohistochemistry for c-Fos, a marker of neuronal activation, to examine the response to α -MSH in pregnant and non-pregnant rats.

Pregnant (n = 6) and non-pregnant (n = 7) rats had indwelling cannulae surgically implanted to allow injections into the lateral cerebral ventricle. On day 14 of pregnancy (or diestrus, in non-pregnant rats), animals were injected with α -MSH (10 μ g) or saline. Ninety minutes later, they were anaesthetised and transcardially perfused, and the brain collected and processed for immunohistochemistry for c-Fos.

In response to α -MSH, non-pregnant rats showed a significant increase in numbers of neurons expressing c-Fos in the arcuate nucleus and ventromedial hypothalamus (VMH) (43 ± 1.5 to 77 ± 14 and 33 ± 5 to 106 ± 7 , respectively) compared with the vehicle controls (ANOVA, Neuman-Kewls post hoc test, $P < 0.05$). Pregnant rats did not show this increase, with no significant change in numbers of neurons expressing c-Fos following α -MSH injection.

These data support our hypothesis that there is a loss of response to α -MSH in the arcuate and VMH hypothalamic nuclei during pregnancy. This is likely to contribute to the state of leptin resistance during pregnancy, facilitating increased food intake and weight gain.

Effects of aging on arginine metabolism in the striatum and spinal cord in rats.
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Aging is a multi-factorial process, and leads to cognitive decline. Recent evidence suggests that altered arginine metabolism in memory-related brain structures contributes to cognitive decline during aging. Arginine, a semi-essential amino acid, is metabolised by nitric oxide synthase (NOS), arginase and arginine decarboxylase (ADC) to produce a number of active molecules. The striatum is the brain region important in reward-association learning and visual-recognition-memory. The present study investigates the effects of aging on the three enzymes involved in arginine metabolism in the striatum, as well as the spinal cord as a comparison.

Male Sprague-Dawley rats, 3 (young, $n = 9$) and 24 (aged, $n = 9$) months old, were euthanised. The anterior and posterior portions of the striatum and cervical spinal cord from each animal were collected. Radioenzymatic and spectrophotometric assays were used to measure the levels of NOS and arginase activities, respectively. The western blot technique was used to determine the protein levels of neuronal NOS (nNOS), endothelial NOS (eNOS), arginase I (AI) and ADC.

There were no significant differences between groups in NOS activity in the anterior or posterior portions of the striatum. However, a significant decrease with age in NOS activity was observed in the cervical spinal cord (Student's t -test, unpaired $t(15) = 3.18$, $P < 0.01$). Arginase activity and the protein levels of nNOS, eNOS, AI and ADC did not differ between the young and aged groups in any region examined.

The present study found no alteration in the activity or protein levels of NOS, AI or ADC with age in the striatum. Interestingly, there was a dramatic decrease in NOS activity with age in the cervical spinal cord. This finding merits further investigation to understand the effects of aging on arginine metabolism in the spinal cord and the functional significance it may hold.

Plasticity-related down-regulation of microRNA regulators of gene expression. B Ryan¹, D Guévremont¹, M Ryan¹, B Logan², W Abraham², J Williams¹. Brain Health Research Centre, ¹Department of Anatomy and Structural Biology, Otago School of Medical Sciences, ²Department of Psychology, Division of Sciences, University of Otago, Dunedin.

The persistence of long-term potentiation (LTP), a widely accepted model for memory, depends on new gene expression. Recently, using microarray expression profiling, our laboratory found that newly-discovered molecules termed microRNA (miRNA) are likely to contribute to the control of gene expression in dentate gyri 20 min after LTP induction in awake rats. The aim of this research was to validate the connection between a select group of these microRNAs and LTP persistence.

This study was undertaken in three parts. First, a literature search was performed to link the potentially LTP-related miRNAs with synaptic activity in previous studies, in order to determine which miRNAs warranted further investigation. Second, real-time reverse transcription-quantitative PCR (RT-qPCR) analysis was used to confirm the microarray data for the miRNAs of interest (n = 4). Finally, we performed real-time RT-qPCR analysis of additional miRNA samples (n = 5) to further pursue the relationship between the miRNAs of interest and LTP.

The literature search revealed that three of the miRNAs that were differentially expressed in the microarray had previously been linked to synaptic plasticity: miR-132, miR-181c and Let-7d. RT-qPCR confirmed that miR-132 was down-regulated by 0.77 ± 0.06 fold (mean \pm SEM, $P = 0.03$ one-tailed t -test; n = 4). However, using the current technology, miR-181c and Let-7d were not found to be differentially expressed ($P > 0.05$; n = 4).

MiR-132 regulates dendritic morphogenesis in an activity-dependent manner by decreasing synthesis of p250GAP, which results in activation of an actin remodelling pathway. Thus, regulation of miR-132 in response to LTP, as observed in our study, may regulate dendritic morphogenesis, a process thought to contribute to LTP persistence. Our results indicate that miR-132 is a promising candidate for further study of LTP-related gene expression.

Crystallisation and preliminary X-ray diffraction of an ATP-bound Hsp70 from *Escherichia coli*. A Gommans¹, M Mayer², S Wilbanks¹. ¹Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin. ²Centre for Molecular Biology, University of Heidelberg, Germany.

The molecular chaperone Hsp70 mediates protein folding and stability. Malfunction of molecular chaperones leads to failure of proteostasis, contributing to diseases as diverse as cancer and Alzheimer's. The ATP-bound structure of Hsp70 has not been characterised; understanding this form will give crucial insight into how the two domains of Hsp70 interact to protect client proteins in proteostasis. We have produced crystals of a modified Hsp70 bound to ATP and collected X-ray diffraction data for structural characterisation.

Modifications of Hsp70 were inspired by the structure of Hsp110, a distant homologue that is neither a molecular chaperone nor ATPase. Modified Hsp70 lacks

the carboxyl-terminal 33 amino acids, contains both a T199A mutation that abolishes ATPase activity, and two additional cysteines that form a cross-link designed to lock the two domains of Hsp70 together in the ATP-bound state. Modified Hsp70 was expressed in *Escherichia coli* BB1553 (Δ DnaK).

Vapour diffusion hanging-drop crystallisation was used to produce crystals from polyethylene glycol. Diffraction data were collected on beamline MX2 at the Australian Synchrotron. Their space group is C_{121} , with unit cell parameters $a = 203 \text{ \AA}$, $b = 78 \text{ \AA}$, $c = 183 \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 102^\circ$, $\gamma = 90^\circ$. The Matthews coefficient of $2.68 \text{ \AA}^3/\text{Da}$, indicates 4 or 5 copies of Hsp70 per asymmetric unit in the crystal. Diffraction extended to 2.8 \AA , with moderate intensity (overall I/σ of 7.2, I/σ of 2.0 at the highest resolution). Data were 99.7% complete and were collected with average multiplicity of 7.0. Merging multiple measurements of each reflection gave an R_{merge} of 0.20 overall (0.88 at the highest resolution).

The statistics indicate that larger crystals are required for a high resolution structure, but that the available crystals are sufficient to solve a low resolution model of the conformation of Hsp70 in complex with ATP.

The cardiac response to β -adrenergic stimulation is reduced in the obese rat heart: the role of adenosine monophosphate-activated protein kinase (AMPK). A. Thaug, R Lamberts. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.

During surgery, patients with obesity have increased incidence of hypotension, requiring higher dosage of catecholamines (adrenaline). This may be responsible for their increased cardiovascular complications. However, the specific effects of catecholamines on blood pressure and its signaling pathway appear to differ in obesity. Adenosine monophosphate-activated protein kinase (AMPK), a key regulator of cardiac energy metabolism, contributes to the disturbed metabolic regulation in obesity. Therefore, the aim of this study was to determine the cardiac response to β -adrenergic stimulation in the obese rat heart and investigate the involvement of AMPK.

Isolated Langendorff-perfused hearts of sixteen-week-old male lean and *fa/fa* obese Zucker rats ($n = 24$) were used to determine cardiac function. Normalised percentage of left ventricular developed pressure was used to quantify the response to adrenergic stimulation. All the hearts were exposed to accumulating doses of β -agonist, isoproterenol (10^{-10} to 10^{-7} M), followed by random assignment to either AMPK antagonist, compound C (CC) ($10 \mu\text{M}$), or Krebs-Henseleit buffer for control group.

In the isolated hearts of lean and obese Zucker rats, no significant differences in basal cardiac characteristics were observed. However, the response to β -adrenergic stimulation was depressed in the obese compared with the lean group, indicated by increased EC_{50} (half maximal effective concentration) values (lean vs. obese: -8.53 ± 0.04 vs. -8.38 ± 0.05 (mean \pm SEM); $P < 0.05$, two-way ANOVA followed by a Bonferroni post-hoc test). AMPK inhibition significantly decreased sensitivity to adrenergic stimulation in lean and obese (lean vs. lean + CC: -8.53 ± 0.03 vs. $-8.21 \pm$

0.03; obese vs. obese + CC: -8.34 ± 0.05 vs. -8.15 ± 0.06 , both $P < 0.05$) diminishing the difference in β -sensitivity between both groups.

AMPK is involved in the cardiac adrenergic stimulation and may be responsible for the attenuated cardiac response to adrenergic stimulation in the obese heart.

Human papillomavirus virus-like particles and their potential as gene delivery vectors in the skin. C Burn, J Leong, M Hibma. Virus Research Unit, Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, Dunedin.

Human papillomavirus (HPV) infection has been causally linked to the development of cervical cancer and, more recently, head and neck cancers. Virus-like particles (VLPs), morphologically and immunologically similar to the natural virion, have been developed to vaccinate against HPV infection of the skin. VLPs, made up of the L1 and L2 capsid proteins of HPV, self-assemble into virions that encapsidate plasmid DNA. The purpose of this study was to generate HPV VLPs that can be used as a delivery vector for DNA vaccines in the skin.

The production and gene delivery capability of HPV VLPs was tested using VLPs containing red fluorescent protein (RFP) or the model antigen, ovalbumin (OVA). 293TT cells were transfected with plasmids containing the genes for L1, L2 and RFP or OVA. The resultant VLPs were used to transduce 293TT cells in culture or carry out *in vivo* assays. OT-1 CD8⁺ T cells that proliferate in response to the OVA peptide were used as a measure of the cytotoxic T cell response. OT-1 proliferation following the subcutaneous immunisation of mice with OVA peptide was compared with epidermal and subcutaneously administered VLPs, and an untreated control (n = 8 for all groups).

VLPs were successfully assembled in 293TT cells, as confirmed by transmission electron microscopy. The VLPs efficiently transduced 74% to 100% of cells in culture. Expression of the gene of interest was detected by fluorescence *in vitro* and by OT-1 proliferation in mouse models. Dermal abrasion prior to administration of VLPs into the epidermis was found to induce significantly greater proliferation of OT-1 cells than subcutaneous delivery of the VLPs (14% versus 10%, $P = 0.0458$, Student's *t*-test).

The ability of HPV VLPs to deliver a gene of interest *in vivo* provides evidence for the potential usefulness of the vectors in the administration of DNA vaccines.

Pulmonary hypertension is accentuated in heart failure – assessed using synchrotron radiation microangiography. M Beard, D Schwenke, E Gray, I Campillo. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.

Patients with chronic heart failure (CHF) often develop pulmonary hypertension (PH) due to the impact left ventricular failure has on the pulmonary circulation. Dysfunction of the pulmonary endothelium acts as a trigger in many etiologies of PH. To date, the role of the endothelium during the onset of CHF-induced PH is unclear.

This study assessed pulmonary endothelial function in a rat model of CHF and determined whether endothelial dysfunction is a leading contributor to the onset of PH.

Dahl-salt sensitive rats (D-Sen, n = 7, CHF-model) and Dahl-salt resistant rats (D-Resis, n = 7) were used. However, since both groups were subjected to a high salt diet for 6 weeks prior to experimentation, Sprague Dawley rats were the control (n = 7). Rats were anaesthetised (pentobarbital, 60 mg/kg) and utilising synchrotron radiation microangiography, we assessed changes in vascular responsiveness to i) acetylcholine (ACh, 3.0 µg/kg/min for 5 min), an endothelium-‘dependent’ vasodilator, ii) the nitric oxide (NO) donor sodium nitroprusside (SNP, 5.0 µg/kg/min for 5 min), an endothelium-‘independent’ vasodilator, iii) endothelin-1 (ET-1, 1 nmol/kg), a vasoconstrictor and iv) BQ-123 (1 mg/kg), an ET-1_A receptor antagonist.

The dilatory response to ACh was impaired in D-Sen and, partly, in D-Resis rats (internal diameters (ID) increased $11.1 \pm 1.7\%$ and $13.8 \pm 1.9\%$, respectively) compared with an $20.8 \pm 2.2\%$ increase in controls (two-sided *t*-test, *P* < 0.05). The vasodilatory responses to SNP, however, were similar for all groups (ID increased ~17%). The vasoconstrictor response to ET-1 was accentuated in all Dahl rats, although the vasodilatory response to BQ-123 was enhanced only in D-Sen rats compared with D-Resis and control rats (ID increased by $15.3 \pm 4.3\%$, $4.9 \pm 2.1\%$ and $8.8 \pm 1.5\%$, respectively, *P* < 0.05).

In conclusion, these results demonstrate that in CHF, endothelial dysfunction, which impairs NO release and enhances ET-1 sensitivity, plays a significant role in the secondary development of PH.