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Abstract Book

Does size matter: preliminary observations on the influence of cadaver size on decomposition rate, insect assemblage and soil chemistry in the summer season of Cape Town, South Africa

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Presentation Slot: PS1

Abstract:

Introduction

The role that cadaver size plays on the rate of decomposition, insect assemblage and soil chemistry has been controversial. In the Western Cape Province and Table Mountain region, no studies have examined if there are differences on the rate of decomposition, insect assemblage and changes in soil chemistry underneath decomposing neonate and adult-sized cadavers. Therefore, we compared the decomposition rate, insect assemblage and soil chemistry data generated from field trials on neonate and adult pig cadavers in the summer seasons between 2020 – 2023 within the Table Mountain National Park.

Materials and Methods

Six neonate pig (~1.3kg) [two (2) for each summer month between 2020/2021] and one adult pig (~60kg) cadavers were used for the study. Information on the decomposition rate of the neonate and adult pig cadavers, insect assemblage and soil chemistry were collected daily using manual photography, purpose-designed insect traps, and a Thermoscientific multimeter respectively.

Results

The time to skeletonization (TTS) between the neonate (mean TTS = 8.8 Days) and adult pig (TTS = 8 Days) cadavers were similar. The adult pig cadaver attracted significantly higher numbers of insect individuals. However, carcass size had no significant effect on the pH and electrical conductivity of the underlying soil.

Discussion

Cadaver size exerted considerable differences mostly on the number of insect individuals and not the decomposition rate or soil chemistry. This may be attributed to the lack of significant differences in ambient temperature, which is the most important non-living driver of decomposition, during the neonate and adult pig summer seasonal trials.

Distinct T cell functional profiles in unvaccinated SARS-CoV-2 seropositive and seronegative children associated with endemic human coronavirus HKU1 cross-reactivity.

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Presentation Slot: PS2

Abstract:

Introduction: Children infected with SARS-CoV-2 are more likely to exhibit asymptomatic or mild disease compared to adults. The immune mechanisms for differences in disease progression between children and adults is not fully understood. Studies assessing SARS-CoV-2 immune responses of paediatric populations in Africa are still scarce. We investigated SARS-CoV-2-specific T-cell responses and functional profile in SARS-CoV-2 seropositive and seronegative unvaccinated children. We also compare the magnitude of the T-cell response between SARS-CoV-2 seropositive children and COVID-19 convalescent adults.

Methods: Children (n=71) aged 0.3-15 years and convalescent adults (n=30) aged 23-58 years, were recruited from Western Cape. Indirect ELISA was used to measure SARS-CoV-2 spike and nucleocapsid-specific IgG, to characterise the cohort serologically. SARS-CoV-2-specific CD4+ and CD8+ T-cell responses were measured using flow cytometry.

Results: 58% of children were seropositive for SARS-CoV-2, indicating past infection. Expectedly, 83% (34/41) of seropositive and interestingly 60% (18/30) of seronegative children had detectable SARS-CoV-2-specific CD4+ T-cell responses. Moreover, SARS-CoV-2-specific CD8+ T-cell responses were detectable in seropositive (49%) and seronegative (47%) children. SARS-CoV-2 seropositive children exhibited a higher proportion of polyfunctional cells compared to their seronegative counterparts. Notably, the frequency of SARS-CoV-2-specific CD4+ T-cells in SARS-CoV-2 seronegative children was moderately associated with HCoV-HKU1 spike-specific IgG. Finally, SARS-CoV-2 seropositive children had a significantly lower magnitude T-cell response compared to convalescent adults.

Conclusion: A large proportion of seronegative children had detectable albeit monofunctional SARS-CoV-2-specific T-cells. This together with the correlation with HCoV-HKU1, may indicate cross-reactivity to endemic coronaviruses, which could contribute to the generally positive clinical outcomes of COVID-19 in children.

Elevated KSHV VL is associated with systemic HIV-related illness in Kaposi's sarcoma patients in South Africa

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Presentation Slot: S1.1

Abstract:

Introduction: Kaposi's sarcoma (KS) is an AIDS-defining illness caused by Kaposi's Sarcoma-associated Herpesvirus (KSHV) in the context of HIV immune suppression.

Materials and Methods: We retrospectively assessed a cohort of KS patients (n=94) receiving treatment at Groote Schuur Hospital for association of KSHV viral load (VL) with staging, response to treatment and outcome.

Results: All patients were HIV positive adults (median CD4 count at diagnosis 186 (IQR: 55–341)), with the majority (99%) on HAART at the time of KS diagnosis. KSHV VL was detectable in the blood of 65 patients (median 280.5/106 cells (IQR: 69.7–1727.3)) and was highest in patients with S1 HIV-related systemic disease (median 1066.9/106 cells, IQR: 70.5–11269.6). KSHV VL was statistically associated with S1 stage in a binomial logistic regression controlling for sex, age, treatment stage and CD4 count (odds ratio 5.565, 95% CI: 1.288–24.041, p=0.021). Six patients with extremely high KSHV VLs (classified as statistical outliers) were predominately T1, I1 and S0 staged with gastrointestinal or pulmonary site of disease and were mostly LTFU or had died at follow-up.

Discussion: Elevated KSHV VL is associated with systemic HIV-related illness in KS disease although our study cannot differentiate between raised VL causing disease progression and severe disease causing KSHV reactivation. A subset of patients with extremely high KSHV VLs and severe clinical features highlights that further investigation into the usefulness of KSHV VL is warranted in identifying patients who require intensive treatment and investigation for progression or diagnosis of concurrent KSHV lytic syndromes.

To investigate whether VEGF and KDR polymorphisms are associated with chronic midportion Achilles tendinopathy and self-reported measurements of tendon pain

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Presentation Slot: S2.6

Abstract:

Introduction: Achilles tendinopathy (AT) is highly prevalent in the general sporting population; modulated by intrinsic and extrinsic factors causing individuals to experience pain. Unlike other types of chronic pain, chronic AT pain may be largely local or peripheral, further explained by the biochemical and/or structural changes in the tendon. The study identified whether VEGFA and its receptor KDR were associated with chronic midportion Achilles tendinopathy and pain.

Materials and Methods: A case-control study was conducted among individuals presenting with AT (n=185) against controls (n=194). Each participant consented to give 5ml of blood, which was genotyped (TaqMan) for the VEGFA polymorphisms, -2758 rs699947 (C/A) and -364 rs2010963 (G/A), while KDR polymorphisms included that of -604 rs2071559 (G/C) and +1719 rs1870377 (T/A) using standard genotyping protocols. Statistical analyses were applied and accepted at $p<0.05$.

Results: Genetic association analyses of polymorphisms among a combined cohort identified significant differences in age, weight and BMI. The CON group was significantly younger ($p = 0.010$) compared to the TEN group at the time of their reported first injury. When covaried for age at the time of recruitment, the CON group, as well as only the first published cohort, were significantly lighter ($p = 0.01$), with a corresponding lower BMI ($p<0.01$), at the time of recruitment comparted to their respective TEN groups. There were noted pseudo-haplotypes among the VEGFA rs699947 and KDR rs2071559 polymorphisms among the combined (C-G; $p = 0.015$) and Severe (C-G; $p = 0.035$) tendinopathy groups.

Discussion: This dissertation expanded the current knowledge on the genetic regions contributing to chronic AT and the pain associated with this condition.

Investigating the co-operation between the human papillomavirus (HPV) oncoproteins E6/E7 with the oncogenic T-box transcription factor 3 (TBX3) to promote cervical cancer.

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Presentation Slot: PS3

Abstract:

Introduction:

Cervical cancer (CC) is a leading cause of cancer-related deaths in women in low- and middle-income countries. Persistent infection with high-risk strains of the Human Papillomavirus (HPV) is the causative agent of CC and its oncoproteins, E6/E7, cooperate with host factors to induce and maintain CC. Therefore, identifying these host factors may have important therapeutic benefits. We have previously reported that TBX3 is upregulated in CC tissues and cell lines where it co-operates with E6/E7 to promote CC proliferation and migration. This study explores the E6/E7/TBX3 axis in CC.

Materials/Methods:

E6/E7 were either overexpressed or depleted in CC cells and the effect on TBX3 assessed by qRT-PCR, luciferase reporter assays and western blotting. Co-immunoprecipitation and GST-pulldown assays were performed to determine if TBX3 interacts with E6/E7 and the region(s) of TBX3 involved. Mass spectrometry was performed to identify E6/E7/TBX3 interacting partners and those of interest were validated by co-immunoprecipitation assays.

Results:

This study reveals that E6/E7 activates TBX3 as overexpressing or depleting E6/E7 resulted in a corresponding change in TBX3. We further show that, E6/E7 cooperate with c-Myc to activate the TBX3 promoter. Co-immunoprecipitation analysis revealed that E6/E7 cooperates with the TBX3 DNA binding domain, and E6/E7 alter the TBX3-associated proteome. Indeed, we identified the serine/threonine phosphatase, PP2A, as an E6/E7/TBX3 cofactor, which plays a context-dependent tumour suppressor and tumour promoter role.

Discussion:

The E6/E7/TBX3 axis is important in driving CC progression. Understanding the molecular mechanisms underpinning CC is important in identifying potential therapeutic targets to treat this neoplasm.

Enhancing Immune Recognition and Clearance of Oncogenic HPVs with Surfactant Protein A

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Presentation Slot: PS4

Abstract:

Cervical cancer, primarily caused by oncogenic human papillomavirus (HPV) infection, disproportionately affects low- and middle-income countries (LMIC). Coinfection with Human Immunodeficiency Virus (HIV) exacerbates HPV-related risks. Prophylactic vaccines exist but are limited in LMIC due to cost and access challenges. Affordable strategies are essential for HPV prevention and treatment. Enhancing the innate immune response against HPV is promising due to the virus's immune evasion tactics. Surfactant protein A (SP-A), known for its role as a lung opsonin, has demonstrated inhibition of non-pulmonary pathogens. Previous studies noted SP-A's interaction with HPV16 pseudovirions (HPV16-PsVs), increasing uptake by murine immune cells.

Materials and Methods:

The study investigated oncogenic HPV (types 16, 18, 31, 45, 55, and 58) agglutination and uptake enhancement when exposed to SP-A. Human monocyte-like cells, THP-1, THP-1-derived macrophages, and immature dendritic cells were utilised to assess HPV-PsVs uptake. An in vitro co-culture model of epithelial and immune cells evaluated viral infection.

Results:

The study revealed substantial HPV agglutination with preincubated SP-A. Enhanced HPV uptake was observed in THP-1 cell line, macrophages, and dendritic cells when SP-A was present. In the co-culture model, SP-A-coated HPV-PsVs significantly reduced viral infection.

Discussion:

The findings suggest that SP-A opsonisation leads to heightened immune cell activation and viral clearance, proposing SP-A's potential for topical microbicides against HPV. Ongoing research aims to validate SP-A's protective role against diverse HPV types. By enhancing innate immunity, SP-A offers a promising avenue for addressing the challenge of HPV infection in resource-limited settings.

Modulation of the riboflavin biosynthesis pathway in mycobacteria

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Presentation Slot: PS5

Abstract:

Introduction

Riboflavin (vitamin B2) is the precursor of the flavin coenzymes, FAD and FMN, which play a central role in cellular redox metabolism. Certain microbes, including Mycobacterium tuberculosis (Mtb), can biosynthesize riboflavin de novo. Importantly, riboflavin precursors have been implicated in the activation of mucosal-associated invariant T (MAIT) cells which recognize microbial metabolites derived from the riboflavin biosynthesis pathway.

Materials and Methods

To investigate the biosynthesis and function of riboflavin and its pathway intermediates in mycobacterial metabolism, physiology and for MAIT cell recognition, we constructed conditional knockdowns (hypomorphs) in riboflavin biosynthesis and utilization genes in Mycobacterium smegmatis (Msm) and Mtb by inducible CRISPR interference and analyzed the impact of gene silencing on viability, and on the levels of expression of riboflavin pathway genes, in addition to the levels of the pathway proteins as well as riboflavin in Msm.

Results

Despite lacking a canonical transporter, we showed that both organisms can import and assimilate exogenous riboflavin when supplied at high concentration. We demonstrated functional redundancy in lumazine synthase activity in Msm and found that silencing of ribA2 or ribF was profoundly bactericidal in Mtb. In Msm, ribA2 silencing resulted in concomitant knockdown of other pathway genes coupled with RibA2 enzyme and riboflavin depletion and was also bactericidal.

Discussion

In addition to their use in genetic validation of potential drug targets for tuberculosis, the collection of hypomorphs created here provides a useful resource for further investigation into the role of pathway intermediates in MAIT cell recognition of mycobacteria.

Exploration of oxidative stress markers in type 2 diabetes mellitus

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Presentation Slot: PS6

Abstract:

Introduction: Type 2 Diabetes Mellitus (T2DM) is a prevalent metabolic disease characterised by chronic hyperglycaemia. Intracellular hyperglycaemia results in oxidative stress due to reactive oxygen species (ROS). Polyunsaturated fatty acids on cellular membranes are vulnerable to ROS damage, triggering lipid peroxidation which produces conjugated dienes (CDs) and lipid hydroperoxides that decompose to form products like malondialdehyde (thiobarbituric acid reactive substance (TBARS)). Chronic oxidative stress produces products which may be found on membranes or are released. The principal objective of this study was to investigate the associations between the concentrations of lipid peroxidation products and T2DM progression.

Materials and methods: Ninety patients (56 women) aged 53±14 years with T2DM were recruited. HbA1c levels and anthropometrics were recorded. Spectrophotometric assays determined the concentrations of CDs and TBARS, which were normalised to phospholipid concentration, in plasma and lipids extracted from erythrocyte membranes.

Results: Male participants had lower HbA1c levels (8.8±1.8%) compared to females (9.7±2.3%), ($P<0.001$). The concentration of plasma normalised CDs in females (2.73±0.6 µmol/mmol) was lower than in males ($P=0.0087$). There was a positive correlation between plasma normalised CDs and participant age ($R=0.228$, $P=0.040$). There was also a positive correlation between TBARS, and CDs extracted from erythrocyte membranes ($R=0.499$, $P<0.001$). There was an upper quartile correlation between HbA1c and plasma normalised TBARS ($R=-0.553$, $P=0.011$) with no other significant associations observed between HbA1c or BMI and oxidative stress markers.

Discussion: Our findings indicated a correlation between TBARS and CDs with males having higher levels of normalised plasma CDs despite better glycaemic control.

Therapeutic Evaluation of CD64-based Immunotherapeutics towards CD64+ cells and ex-vivo differentiated macrophages.

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Presentation Slot: S3.1

Abstract:

Introduction:

Dysfunctional macrophages are involved in the pathogenesis of chronic inflammatory diseases and cancers. Currently, there is a medical need to replace symptomatic agents with novel curative agents that can eliminate these dysfunctional cells. Leveraging immunotherapy, a single chain variable fragment (scFv) of an antibody recognizing the overexpressed Fc gamma receptor 1 (FcγRI or CD64) on dysregulated macrophages, H22(scFv) was used to specifically deliver a cytotoxic payload (toxin/drug/theranostic) for precise diagnostic and therapeutic outcomes. The CD64-based immunotherapeutics evaluated include recombinant immunotoxins, rITs (H22 linked toxins): H22-ETA, and its deimmunized version; H22-dETA; and SNAP-tag ADC (H22 linked drug): H22-AURIF, and PIC (H22 linked theranostic photosensitizer): H22-IR700.

Materials/Methods:

In silico cloning and molecular cloning of plasmids expressing CD64-fusion proteins into their hosts of expression (rITs = bacterial (BL21 E. coli), while SNAP-tag proteins = mammalian (HEK293T cells)). Recombinant proteins were harvested and purified with a mix of chromatographic techniques (IMAC, SEC). Structural characterization was confirmed with SDS-PAGE, Western blot, and Quantitative densitometry. Lastly, functional assays including antigen-dependent binding and in vitro cytotoxicity studies towards CD64+ cells (IFNγ- U937 and HL-60) and ex-vivo differentiated macrophages were conducted.

Results/Discussion:

Functional rITs – H22-ETA and H22-dETA demonstrated significant antigen-specific binding and cytotoxicity towards CD64+ cells in nanomolar dosage. Also, M1 pro-inflammatory macrophages with upregulated CD64 demonstrated significant internalization of H22-SNAP proteins. ADC, H22-AURIF demonstrates cytotoxicity towards CD64+ proliferating cells while PIC, H22-IR700 induced necrosis significantly in MI proinflammatory macrophages. These CD64-targeting immunotherapeutics showed therapeutic potential and could serve as precision therapy for macrophage-mediated diseases.

ABSTRACT WITHDRAWN

The role of genetic signatures within the ABCB1, ORPM1 and COMT genes in the development of chronic shoulder pain and shoulder disability in breast cancer survivors

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Presentation Slot: PS7

Abstract:

Introduction: Chronic pain/disability is a major side effect observed in breast cancer survivors (BCS) post-treatment. Standard treatment for acute and chronic pain, mainly relies on opioid/derivatives. One emerging risk factor currently being investigated is the role of genetics, particularly the ABCB1, OPRM1, and COMT genes that form part of the opioid signaling response pathway. Polymorphisms within these genes have been implicated in pain variability. This study aimed to investigate the genetic association between ABCB1, OPRM1, and COMT SNPs and chronic pain/disability in BCS. Materials and Methods: A cross-sectional study was conducted with BCS >18yrs, diagnosed >1yr prior and of mixed ancestry. The Shoulder Pain and Disability Index (SPADI) was used to measure patient-reported pain, disability, and combined symptoms. N=252 BCS samples were genotyped(TaqMan), for ABCB1(rs1045642:G>A), OPRM1(rs1799971:A>G), and COMT(rs4680:G>A). Using individual SNP genotype data, inferred allele-allele combinations were assessed as a proxy for gene-gene interactions. Statistical significance was accepted at $p<0.05$. Bioinformatic analyses were conducted to explore gene-associated networks. Results: The G-A-A allele combination was significantly associated with increased likelihoods of reporting moderate-high pain (p=0.001,OR:1.93,95%CI:1.01-3.69,HS=3.20) and combined (p=0.002,OR:1.60,95%CI:0.81-3.19,HS=3.17) symptoms. In contrast, the G-G-G allele combination was significantly associated with increased likelihood of reporting no-low pain (p=0.014,OR:1.00,95%CI:1.00-1.00,HS=-2.50). While the A-A-G allele combination was significantly associated with an increased risk of reporting no-low disability (p=0.015,OR:1.37,95%CI:1.13-2.01,HS=-2.43) and combined (p=0.014,OR:1.51,95%CI:1.22-2.20,HS=-2.47) symptoms. Discussion: The findings of this study suggest that collectively, the sequence signatures within key gene regions of ABCB1, OPRM1, and COMT, may contribute to the development of chronic shoulder pain/disability in BCS.

Effects of Acupuncture Treatment on Resting-State Functional Connectivity in Stroke Patients with Unilateral Limb Dysfunction

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Presentation Slot: S2.1

Abstract:

Stroke patients often endure extended hospitalisation and lasting physical and cognitive impairments of varying severity. Acupuncture treatment has the potential to stimulate the central nervous system and aid functional recovery. Resting-state fMRI (rs-fMRI) identifies brain regions with temporal correlation during task-free states. The aim of this study was to evaluate the potential benefits and neural mechanisms of acupuncture treatment in ischaemic stroke patients with unilateral limb dysfunction. Resting-state functional connectivity (RSFC) was used to quantify the temporal correlation among distinct neuronal activations.

Participants (5 stroke patients, 10 healthy controls) were recruited from the Tianjin University of Traditional Chinese Medicine. Two sets of MRI data were acquired on the 5 stroke patients pre- and post-acupuncture treatment. One set of MRI data was acquired on the controls without any treatment. Data preprocessing employed afni_proc.py. Ten resting state networks (RSNs) were identified from pre- and post-acupuncture treatment MRI data by group independent component analysis (ICA). Clusters within the RSNs showing significant differences post- vs pre- acupuncture treatment were identified. Mean z-score, a measurement of RSFC, within each cluster was calculated and was correlated to the Fugl-Meyer Assessment (FMA), a measure of post-stroke body function impairment.

Post-acupuncture treatment, significantly higher RSFC was observed in the cerebellum and basal ganglia networks, correlating with increased FMA compared to pre-acupuncture z-scores. No substantial differences emerged in comparing post-acupuncture RSFC of stroke patients to healthy controls. The substantial rise in RSFC and FMA signifies acupuncture's potential to enhance RSFC and mobility among stroke patients.

Impact of intrauterine HIV exposure on infant antiviral antibody repertoire

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Presentation Slot: PS8

Abstract:

Introduction

Antibody responses to vaccines do not explain health outcomes disparities observed between infants who are HIV-exposed but uninfected (iHEU) and HIV-unexposed uninfected (iHUU). We investigated the effect of HIV-exposure on infant humoral immunity by comparing viral-specific antibody repertoires between iHEU and iHUU.

Materials and Methods

IgG against 206 eukaryote-infecting viruses was measured using Phage Immunoprecipitation Sequencing in plasma of iHEU (n=15) and iHUU (n=15) at birth and 36 weeks of life.

Results

High-dimensional reduction analysis revealed age-associated difference in repertoire of virus-targeting antibodies between paired birth and week 36 samples (p=0.001). At birth, antibody repertoires were different between iHEU and iHUU (p=0.002), although they converged by week 36 (p=0.790). iHUU had a greater number of unique viral antibody targets per participant at birth than iHEU (median; 9 vs. 6; p =0.035), although there were no differences by week 36 (median; 7 v.s 6; p=0.500). Antibodies against HIV were detected in 10 and 1 iHEU at birth and at week 36, respectively, showing a >2-fold longitudinal decrease (p=<0.001). Common viral targets at birth included herpes simplex-1 (90.0%) and -2 (73.3%), cytomegalovirus (93.3%), mastadenovirus C (63.3%) and rhinovirus A (96.6%), whereas antibodies against enterovirus B (70.0%) and C (70.0%), cytomegalovirus (76.6%), rhinovirus A (83.0%) and B (60.0%) were common at week 36. Mastadenovirus C antibody levels were higher in iHUU compared to iHEU at birth (mean; 28.06 vs. 24.27; p = 0.048).

Discussion

Lower antibody repertoire breadth of iHEU at birth could have clinical significance including increased risk of infections, warranting further investigations.

Variations in the circulus arteriosus cerebri in a South African cadaveric sample.

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Presentation Slot: PS9

Abstract:

The circulus arteriosus cerebri (CAC), or the Circle of Willis, is a highly variable arterial system at the base of the brain that allows for collateral blood flow. The CAC has been described as having a protective effect during ischaemic events. However, the CAC is not always able to compensate during ischaemic events. Furthermore, some variations of the CAC have been associated with aneurysm formation and subsequent rupture which results in a subarachnoid haemorrhage. Therefore, it is important to know the prevalence of variations in a population in an effort to understand the potential effects of the variations. For materials and methodology, this is a cross-sectional and quantitative study approved by the UCT Human Research Ethics Committee (ref: 645/2022) and Cadaver Research Governance Committee (CRCG 2022/009). The brains of 94 bodies in the Department of Human Biology, UCT, were analysed. Figi® software was used to measure the lengths and diameters of vessels. Patency of hypoplastic vessels was investigated using a dissection microscope. Brain volumes were compared to the vessel diameters to determine if a correction factor for human variation should be applied.

The variations present will be statistically tested for associations with age, sex and the presence of aneurysms. Based on the published literature, hypoplasia or aplasia of the PcoA may be most common, followed by multiplications of the AcoA. Few studies have been conducted in a South African context and it is uncertain how the results of this study will compare with data derived from other local studies.

Effect of neurohumoral stimulation on stem cell- derived cardiac-like cells

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Presentation Slot: PS10

Abstract:

Introduction:

Heart failure is a global pandemic and an economic burden in both developed and developing countries. During heart failure, the sympathetic nervous system (SNS) is hyperactivated to stimulate the adrenergic receptors and maintain cardiac homeostasis. SNS hyperactivity results in the sustained activation of β-adrenergic receptors (β-ARs) in the heart, which might result in cardiac remodeling characterised by molecular, morphological and functional changes. However, the effect of SNS hyperactivation on cardiac remodeling is poorly understood. Therefore, this study aimed to explore the impact of overstimulation with isoprenaline, an analog of the endogenous catecholamine epinephrine, on the molecular, morphological, and functional alterations within an in vitro model of cardiac-like cells derived from mouse embryonic stem cells (mESCs). The investigation focused on two key molecules: GATA4, a zinc-finger transcription factor associated with cardiac remodeling, and cardiac troponin T (cTnT), a marker of cardiac differentiation important for cardiac contractile function.

Methods:

The study was carried out in cardiac-like cells derived from mESCs. Confocal and light microscopy, immunochemical staining and video recordings of cardiac-like beating were used for morphological, molecular and functional analysis, respectively.

Results and conclusion:

The results showed that isoprenaline overstimulation increased the number of pyknotic-like nuclei and decreased GATA4 expression levels compared to control. Additionally, the qualitative analysis showed that cTnT was reduced in the isoprenaline-overstimulated group compared to control. Furthermore, cardiac contractile dysfunction, characterized by a significant increase in the beating rate and decreased amplitude was observed in the isoprenaline-overstimulated group.

Assessing the applicability of the pulp/tooth area ratio (PAR) method for estimating adult age-at-death in a South African cadaveric sample: A comparative study using maxillary canine periapical radiographs and tooth section images

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Presentation Slot: PS11

Abstract:

Introduction:

Establishing age-at-death is essential in biological anthropology. Current conventional techniques employed for South African adults are deficient and unable to provide satisfactory estimates, emphasising the need for additional methods. Radiographic and sectioning (stereomicroscopic) techniques were employed to evaluate the applicability of the pulp/tooth area ratio (PAR) ageing method when applied to maxillary canines from a South African cadaveric sample.

Materials and Methods:

Following extraction and cleaning, 52 adult, cadaveric teeth were subject to radiographic and stereomicroscopic procedures. Labiolingual and mesiodistal radiographs and tooth section images were obtained. Images were analysed using ImageJ to obtain PARs. Models were derived for each image type (including and excluding enamel area) and compared for their performance and accuracy for age-at-death estimation.

Results:

Stereomicroscopic tooth section image models demonstrated the highest performance and accuracy, obtaining mean absolute errors (MAEs) and standard error of the estimates (SEEs) of 7.45 - 7.72 years and 10.17 - 10.84 years respectively. Labiolingual radiographic models (MAEs = 9.89 - 10.13 years; SEEs = 12.32 - 12.45 years) performed better than mesiodistal radiographic models (MAEs = 12.31 - 12.37 years; SEEs = 15.64 - 15.85 years). Models excluding enamel area performed better.

Discussion:

The PAR method represents the most accurate dental ageing technique assessed for South African adults. The (labiolingual) radiographic approach will be useful in anthropological investigations as it is relatively accurate, minimally invasive, inexpensive and applied rapidly. These results support its use as an additional age indicator to complement, inform and enhance current anthropological age estimation analyses in South Africa.

Pharmacogenomics of Tamoxifen: The Role of Genetic Variation in Phase II Pharmacogenes on Tamoxifen Response among Breast Cancer Patients in South Africa

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Presentation Slot: S1.4

Abstract:
Introduction: Tamoxifen is the standard hormone therapy used to treat oestrogen receptor-positive breast cancer and effectively reduces recurrence and mortality. Nevertheless, the response to tamoxifen treatment varies among patients, with up to 50% of patients experiencing a recurrence. This variability in response can partly be attributed to genetic variation in genes encoding enzymes involved in tamoxifen metabolism. The pharmacogenetics of tamoxifen in populations of African descent remain understudied, creating difficulties in pinpointing the primary factors behind the observed variable response. To address this gap, this study aimed to investigate the effects of genetic variation in genes within two major phase II families, UDP-glucuronosyltransferases (UGTs) and sulphotransferases (SULTs), due to their central role in tamoxifen disposition.
Materials and Methods: The study included 185 breast cancer patients who had undergone tamoxifen treatment. Genetic characterisation was done for 39 single nucleotide polymorphisms (SNPs) in nine genes, UGT1A4, UGT1A8, UGT1A10, UGT2B7, UGT2B15, SULT1A1, SULT1A2, SULT1E1, and SULT2A1, using various methods. Odds ratios were calculated on the associations between genotypes and either 5-year relapse-free survival (RFS) or overall RFS.
Results: Some SNPs in UGT1A4, UGT1A10, and SULT1A1 were significantly associated with both 5-year and overall RFS. Moreover, variants within SULT2A1 were found to significantly influence 5-year RFS.
Discussion: These results highlight the role of genetic variation within phase II pharmacogenes in shaping the outcomes of tamoxifen therapy among South African breast cancer patients.

Regulation of lymphomagenic factor AICDA by miRNA-181b in HIV-associated aggressive B-cell Lymphomas.

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Presentation Slot: PS12

Abstract:

INTRODUCTION

Non-Hodgkin lymphoma (NHL) constitutes a diverse group of aggressive malignancies, with the subtypes Diffuse Large B-cell Lymphoma (DLBCL) and Burkitt Lymphoma being associated with Human Immunodeficiency Virus (HIV) infection. Overexpression of Activation-induced cytidine deaminase (AICDA/AID), a DNA-modifying enzyme, is a driving factor of these cancers. Investigations of the dysregulation of AICDA/AID in NHL may provide useful insights into disease aetiology. This study defines the regulation of AICDA by the microRNA-181b.

MATERIALS AND METHODS

Luciferase reporter assays and site-directed mutagenesis will be used to study the regulation of AICDA by miRNA-181b via the 3`-UTR. Western Blotting and q-PCR will be used to assess changes in AICDA/AID expression in lymphoma cell lines ectopically expressing a FAM-labelled miRNA-181b mimic. q-PCR will be used to assess and compare expression profiles of miRNA-181b and AICDA in the peripheral blood mononuclear cells (PBMCs) of newly diagnosed, chemotherapy-naïve HIV-positive and HIV-negative DLBCL patients.

RESULTS

In silico analysis identified three putative miRNA-181b binding elements within the AICDA 3`-UTR, and reporter assays show that the miRNA is a direct repressor of this gene. MiRNA-181b expression was highly variable in HIV-positive DLBCL patients compared to relatively low and less variable expression in HIV-negative DLBCL patients. Additionally, the HIV protein Tat was found to repress miRNA-181b expression.

DISCUSSION

The results reveal that the human AICDA gene is a direct target of miRNA-181b, and that HIV infection impacts the expression of this miRNA. Current work continues to investigate the HIV-miRNA-181b-AICDA/AID axis in HIV-associated NHLs.

Reactivation of KSHV by co-infections and inflammation in HIV-infected patients from South Africa

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Presentation Slot: PS13

Abstract:

Introduction: In South Africa, high exposure to Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the COVID-19 global pandemic, occurred primarily in densely populated, low-income communities which are additionally burdened by a high prevalence of Human Immunodeficiency Virus (HIV) and Kaposi's Sarcoma Associated Herpes Virus (KSHV). It is currently unknown whether SARS-CoV-2 exposure has long-term impacts on pathologies associated with these two viruses. Therefore, the main aim of our research was to assess SARS-CoV-2 seroprevalence and its association with HIV-related clinical parameters as well as KSHV reactivation in a rather homogenous socio-economic group of patients, largely unprotected and likely to be similarly exposed to SARS-CoV-2.

Materials and Methods: We conducted a cross-sectional study at the Gugulethu Community Health Centre Antiretroviral clinic in the Western Cape of South Africa between September 2020 and April 2023. The period of recruitment was associated with the decline of SARS-CoV-2 infections from the first COVID-19 wave in South Africa and before nation-wide roll-out of vaccinations. A total of 407 non-hospitalised adult (>18 years of age) HIV-infected patients was recruited to the study.

Results and Discussion: The majority of the patient cohort (>95.0%) was on anti-retroviral therapy (ART) with an HIV viral load (VL) ranging from 1 – 1217796 copies/ml. Of the patients tested, >80.0% were found to have had a previous SARS-CoV-2 infection due to positive serology. Interestingly, >50.0% of this cohort indicated a positive KSHV serostatus (K8.1 or ORF73 antibodies). Moreover, preliminary results of this study show an increase in KSHV lytic reactivation due to SARS-CoV-2 infection (but not vaccination) in these non-hospitalised patients without current indication of adverse disease outcome which warrants further follow-up.

Retrospective Audit of Inherited Metabolic Disease Diagnoses: Implications for Newborn Screening in South Africa

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Presentation Slot: PS14

Abstract:

Background

There is no national newborn screening program (NBS) in South Africa, and less than 1% of newborns are screened for any rare disease. Prior research has estimated higher newborn incidences for galactosaemia I (GALT), glutaric acidemia 1 (GA1), neuropathic cystinosis and mitochondrial DNA depletion syndrome 6 (hepatocerebral type) (MPV17) in South Africa compared to other countries. However, a data deficit on rare diseases in South Africa remains and necessitates research on the presence and incidence of IMDs to guide the path towards NBS.

Methods

A retrospective audit was performed on the urine organic acid (UOA) test data from our diagnostic services since October 2015. Data from our IMD database and repository were also included to determine the estimate of cumulative incidence of IMDs and the spectrum of all IMDs, diagnosed by these services between 2015 and 2021.

Results

A total of 9068 requests for UOA testing were received during the study period. The majority (85%) of these profiles were unremarkable, with only 1% of UOA profiles resulting in an IMD diagnosis. One third of the IMD diagnoses made were through genetic testing and the remainder were through biochemical testing.

Discussion

South Africans of African ancestry have amongst the highest disease incidences for GALT, GA1, cystinosis and MPV17 in the world. In the absence of an appropriate NBS program, many patients remain undiagnosed or receive a late diagnosis with preventable morbidity. We argue that a combined molecular/biochemical program may be the most appropriate way to implement NBS in South Africa.

An assessment of the status of the regulatory B cell population (CD45+CD19+CD24++CD38++) and programmed death-ligand 1 positivity (CD45+CD19+CD274++) in the PBMC of HIV-positive and HIV-negative newly diagnosed DLBCL patients at a South African Health care facility.

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Presentation Slot: S1.3

Abstract:

Introduction

The programmed death-ligand 1 (PD-L1) is an important immunoregulatory protein which is overexpressed by tumour cells to promote immune evasion. A subset of PD-L1+ regulatory B-cells (Bregs) has been reported to be associated with the development of HIV-associated Diffuse Large B-Cell Lymphoma (DLBCL), in a study involving a US-based patient cohort. DLBCL is the most common lymphoma worldwide, and the most prevalent non-Hodgkin lymphoma among HIV infected individuals. The current study assesses PD-L1+ Breg status within a cohort of HIV-positive and HIV-negative newly diagnosed DLBCL patients in South Africa.

Methods

Breg (CD45+CD19+CD24++CD38++), PD-L1+ Breg (CD19+CD24++CD38++CD274++), as well as other immune cell populations are measured within the peripheral blood mononuclear cells from newly diagnosed DLBCL patients using flow cytometry. . A sequential gating strategy is used to refine cell populations. The data is grouped according to HIV status and compared using Mann-Whitney U and Kruskal-Wallis statistical tests.

Results

Preliminary analysis performed on seventeen patients, of which 47% were HIV-positive, show more B cells (CD45+CD19+) in HIV-positive patients compared to the HIV-negative group, suggesting that HIV-infection promotes B cell proliferation. Additionally, a higher number of Bregs were detected in the HIV-positive group, relative to the HIV-negative group. A clear difference in the proportion of PD-L1+ Breg cells is not yet clear due to the small sample size, however recruitment is ongoing.

Discussion

The preliminary results have uncovered interesting differences in immune cell populations in HIV-positive and -negative DLBCL patients. Recruitment is ongoing to continue to define the unique pathobiology of HIV-associated DLBCL.

Role of genetic variations of SLCO1B1 and ABCG2 on the occurrence of Statin Associated Muscle Symptoms (SAMS) in a cohort of South African dyslipidemia patients.

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Presentation Slot: S2.4

Abstract:

Introduction: Statin Associated Muscle Symptoms (SAMS) represent a prevalent and serious class of adverse drug effects that frequently lead to the discontinuation of statin therapy in patients with dyslipidemia. There is strong evidence of the association of SAMS with ABCG2 and SLCO1B1 genetic variation, especially in Asian and European populations. This study aimed to elucidate the role of genetic variations of SLCO1B1 and ABCG2 on the occurrence of SAMS in a cohort of South African dyslipidemia patients.

Methods & Materials: We conducted a retrospective matched case-control (1:2) study. Patients with dyslipidemia were recruited from Groote Schuur Hospital, South Africa, with a catchment area for patients of predominantly Mixed Ancestry, Black African, and European ancestry. A lipidologist (DB) screened the medical records of all study participants and assigned SAMS status. A total of 332 participants (110 cases vs 222 controls), had their DNA genotyped for polymorphisms in ABCG2, and SLCO1B1 utilizing TaqMan assays.

Results: The median age of participants was 58 (48-67) years and 50% were female. BMI (P=0.0260), waist circumference (P=0.034), and triglycerides (P=0.006) were significantly associated with SAMS. The ABCG2 rs2231142T variant allele (P=0.034) and genotypes of SLCO1B1 rs4149056 (P=0.020) were significantly associated with SAMS.

Conclusion: The BMI, waist circumference, triglycerides, ABCG2 (rs2231142), and SLCO1B1 (rs4149056) polymorphisms appeared to play a role in SAMS in these South African patients. Therefore, these factors should be considered when addressing SAMS in South African patients with dyslipidemia.

Keywords: Statins, SAMS, Pharmacogenetics, ABCG2 and SLCO1B1.

Repurposing TBX3 targeting drugs niclosamide, piroctone olamine and pyrvinium pamoate for the treatment of cervical cancer

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Presentation Slot: PS15

Abstract:

Introduction: In Africa, cervical cancer (CC) is the second most diagnosed cancer, and it is associated with most cancer-related female deaths. The human papillomavirus encodes two key oncoproteins, E6 and E7, important for the initial establishment and subsequent progression of cervical cancer which it does, in part, through interaction with the T-box transcription factor-3 (TBX3). The Prince group has identified commercially available drugs, niclosamide (NS), piroctone olamine (PO), and pyrvinium pamoate (PP) that target TBX3 to exhibit anti-cancer activity. The anticancer activity induced by NS, PO, and PP in HPV-positive CC was investigated for the first time in the present study.

Methods: HPV-positive CC cells (HeLa and CaSki) were treated with NS, PO, and PP, and the impact on endogenous TBX3 levels was assessed. The effect of the drugs on cell survival (MTT and clonogenic assays), senescence (SA-β-gal/p16), cell cycle (FACS), migration (scratch assay), and spheroid growth (calcein-AM stain) and invasion (collagen matrix) together with the modes of cell death were evaluated.

Results show that NS, PO, and PP inhibited the endogenous TBX3 levels in CC cells and selectively inhibited their short- and long-term survival. Additionally, NS, PO, and PP significantly reduced the spheroid growth, proliferation, invasion ability, induced cell cycle arrests, senescence, DNA damage, apoptosis, and autophagy in a context-dependent manner.

Discussion: Together, these data provide compelling evidence that NS, PO, and PP have potent anti-CC activity and that they may be a potential and affordable anticancer agent that deserves further investigation.

ABSTRACT WITHDRAWN

Evidence of functional connectivity disruptions between auditory and non-auditory regions in children living with HIV

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Presentation Slot: PS16

Abstract:

Introduction: To date, the role of functional connectivity (FC) alterations on auditory function in children living with perinatal HIV (CPHIV) has not been established. We aimed to investigate whether HIV alters FC within the central auditory system (CAS) and between the CAS and non-auditory brain regions.

Methods: Structural and resting-state functional MRI were acquired in 84 11- to 12-year-old children (59 CPHIV). On the structural images, we defined 4 manually-traced bilateral regions of interest (ROIs) along the CAS pathway – the medial geniculate nucleus, a region including the cochlear nucleus and superior olivary complex, inferior colliculus, and primary auditory cortex (PAC), as well as 63 bilateral subcortical and cortical ROIs obtained from automated whole-brain segmentation. RS-FRMI processing in AFNI included motion correction and slice scan time correction; volumes with translation >3mm and/or rotation >3 degrees in any direction were excluded. ROIs were mapped to RS-FMRI and Pearson correlation coefficients (r) computed between the mean time series of each ROI and every other ROI. The resulting FC matrices were compared between CPHIV and HIV-unexposed uninfected children (CHUU) using a Bayesian multilevel model [1,2] which integrates subject across condition and ROI-effect across subject negating the need for multiple comparisons correction.

Results: CPHIV demonstrate lower FC of the PAC, and disruptions in FC between CAS regions and hippocampal sub-regions, the lingual gyri and basal ganglia.

Discussion: These results suggest that HIV-related disruptions in FC within and to the CAS may play a role in the hearing and language impairments seen in this population.

Development of 3D multicellular human liver organoid derived from induced pluripotent stem cells as the tool to model liver diseases

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Presentation Slot: S3.5

Abstract:

Introduction: The liver is an essential organ in the body that perform key functions. Liver diseases and drug cytotoxicity studies have been conducted using cancer-derived cell lines which are inferior. We therefore aimed to develop a robust and tractable multicellular 3D model suitable for studying liver diseases.

Methods: We developed a reproducible method to generate human liver organoids (HLO) from induced pluripotent stem cells (iPSCs). We performed qPCR to confirm stage specific gene markers and validated presence of multiple cell lineages with flow cytometry. We assessed the functionality of the organoids by stimulating HLO with either anti-retroviral drugs or Schistosoma egg antigen (SEA). We analyzed the expression of pro-inflammatory and anti-inflammatory cytokines by Enzyme-linked immunosorbent assay (ELISA).

Results: HLO exhibited robust mature hepatic gene markers (CYP3A4, ATA1, ALB and HNF4-α), reduction in pluripotency (OCT-4, Nanog, and Sox2) and the definitive endoderm (SOX17 and GSC) markers. Flow cytometry using EpCAM, CD166 and CD68 antibodies indicated that HLO comprise 60,4 % EpCAM +, 11,2% EpCAM-/CD166+ and 5% EpCAM+/CD68+ cells, respectively. HLO had high CYP3A4 enzyme activity compared to HepaRG 3D model. Proinflammatory (IL-6, IL-1β and TNF-α) and anti-inflammatory (IL-4 and IL-10) cytokines were elevated in both models. IL-4 was more pronounced in SEA treated HLO only.

Discussion: We successfully generated multicellular 3D liver organoids consisting of hepatocytes, Kupffer cells and hepatic stellate cells. This model could be utilized to examine complex inflammatory liver diseases, elucidate disease mechanisms, and drug toxicity screening.

Selective binding and internalization of SNAP-tag based fusion protein on melanotransferrin/p97-expressing tumors cells lines for an immunotheranostics purpose.

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Presentation Slot: PS17

Abstract:

Cutaneous melanoma is a cancer developing from epidermal pigment-producing cells called melanocytes. Even representing 4% of skin cancer, melanoma accounts respectively for 20% of cancer and 80% of skin cancer related deaths worldwide. More impressively, the rate of melanoma is also increasing in Sub-Saharan Africa countries. Over the last decade, the annual cases have increased by 50% to over 287,000 with around 60,000 melanoma-related deaths. This makes melanoma a silent killer, a growing epidemic worldwide and thus a crucial public health problem. Cutaneous melanoma is the most aggressive and more resistant cancer to current treatments at metastasis stage. Improvements in immunotherapy is giving today greater chances to cure cancers resistant to standard chemotherapy.

Snap-tag based fusion protein, L49(scFv)-SNAP was expressed through mammalian cells lines HEK 293T and purified using Immobilized Metal Affinity Chromatography system. Upon confirmation of the full functionality of L49(scFv)-SNAP, it was labeled to the fluorophore BG-Alexa 488. Afterwards, binding assays were performed on melanotransferrin-expressing tumor cells lines A2058 and MDA-MB 231 using confocal microscopic. HEK 293T cells lines were used as negative control.

Snap-tag based fusion protein, L49(scFv)-SNAP greatly bound and internalized to A2058 and MDA-MB 231, no binding on negative cells lines. The ability of L49(scFv)-SNAP fusion protein to selectively bind to melanotransferrin-expressing tumors cells line, makes it a good candidate for immunotheranostics of melanoma skin cancer once it will be attached/conjugated to a toxin payload to assure both diagnostic and killing of melanotransferrin/p97-expressing tumors cells.

Key words: Melanoma skin cancer, SNAP-tag based fusion protein, Immunotheranostics.

Distinct alterations in white matter properties and organization related to maternal treatment initiation in neonates exposed to HIV but uninfected

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Presentation Slot: S2.8

Abstract:

Introduction: HIV exposed-uninfected (HEU) infants and children are at risk of developmental delays as compared to uninfected unexposed (HUU) populations. The effects of exposure to in utero HIV and ART regimens on the HEU developing brain are not well understood.

Materials and Methods: In a cohort of 2-week-old newborns, we used diffusion tensor imaging (DTI) tractography and graph theory to examine the influence of HIV and ART exposure in utero on neonate white matter integrity and organisation. The cohort included HEU infants born to mothers who started ART before conception (HEUpre) and after conception (HEUpost), as well as HUU infants from the same community. We investigated HIV exposure and ART duration group differences in DTI metrics (fractional anisotropy (FA) and mean diffusivity (MD)) and graph measures across white matter.

Results: We found increased mean diffusivity in WM connections involving the thalamus and limbic system in the HEU-pre group compared to HUU. We further identified reduced nodal efficiency in the basal ganglia, reflecting a decreased ability to send/receive information to/from other network nodes. Within the HEU-post group, we observed reduced FA in cortical-subcortical and cerebellar connections, as well as decreased transitivity in the hindbrain area compared to HUU. Transitivity measures segregation related to specialized processing.

Discussion: Overall, our findings demonstrate distinct alterations in WM integrity related to the timing of maternal ART initiation that influence regional brain network properties.

Forensic DNA profiling on human teeth exposed to marine environments

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Presentation Slot: S2.3

Abstract:

Introduction: DNA recovery from human remains recovered from the ocean is challenging, and there is a lack of empirical research regarding the recovery of DNA obtained over time. Previous research is limited to artificial environments, forensic case reports, and human analogues.

Materials and Methods: In this study we assessed DNA recovery from human teeth exposed to semi-natural and natural marine environments. Sixteen teeth were submerged in tanks, simulating the western coast of Cape Town, with teeth extracted at various intervals over 30 days. Twelve teeth were subsequently submerged in the ocean for 20 days. Control teeth were stored at room temperature for equivalent durations. Extracted DNA was quantified via qPCR and analysed using forensic STR profiling.

Results: For the tank study, a steady decrease in DNA concentration was observed as submersion interval increased. Degradation remained relatively constant throughout. There were no prominent differences in concentration and degradation patterns between the control and the experimental teeth. The quality of forensic DNA profiles diminished as submersion interval increased, and after 16 days of submersion, only partial DNA profiles were obtained. In the natural setting, a significant difference was observed between the control and experimental teeth and no full STR profiles were obtained from experimental samples.

Discussion: Ocean exposure complicates identification and requires significant time, expertise, and context. This research sets a basis for DNA retrieval in marine contexts and provides a baseline measure to suggest when STR profiling might be sufficient and when massive parallel sequencing should be considered in case work, given the post-mortem submersion interval.

Assessment of common metrical sex estimation parameters in southern African Holocene San and Khoekhoe populations.

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Presentation Slot: PS18

Abstract:

Introduction: Accurate population-specific sexing standards do not exist for southern African Holocene San and Khoekhoe populations. Due to their markedly small stature, skeletal gracility, and past physically active lifestyle, this population exhibits reduced sexual dimorphism, complicating application of traditional sexing standards.

Materials and methods: The accuracies of eight common metrical sex estimation variables (six mandibular and two post-cranial epiphyseal) were assessed in 174 archaeological Holocene San and Khoekhoe adults. Sex estimates based on these metrics were compared with pelvic sex estimates (Phenice method) to assess accuracy, and measurements obtained were subjected to cross-validated discriminant function analysis.

Results: All variables assessed were sexually dimorphic, with femoral and humeral vertical head diameter (FHD and HVHD) showing the largest differences. The most accurate discriminant function equations were for direct multivariate mandibular bicondylar breadth, FHD and HVHD (77%), univariate FHD (75%), and stepwise multivariate FHD and HVHD (73%). Minimal accuracy decreases were observed when equations were cross-validated, confirming their predictive accuracy. The least reliable measurements for estimating sex were found to be univariate mandibular bicoronoid breadth (62%) and coronoid height (65%).

Discussion: Whilst all variables were sexually dimorphic, they produced lower accuracy rates than in other populations using the same parameters, illustrating this population's reduced range of sexual dimorphism. Notably, the equations produced here are the first for archaeological San and Khoekhoe people. Application of these equations allows for sex estimation with considerable accuracy and a known error rate, previously not possible for this population.

Cooperation of Epstein-Barr Virus and Hiv in the Pathogenesis of Hiv-Associated Burkitt Lymphoma

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Presentation Slot: PS19

Abstract:

BACKGROUND: Burkitt lymphoma (BL) is overrepresented among HIV-infected individuals, and therefore of high incidence in South Africa, relative to the rest of the world. The c-MYC oncogene translocation, driven by activation-induced cytidine deaminase (AICDA/AID), is a defining feature of BL. Infection with the Epstein-Barr virus (EBV) is linked to AID deregulation and onset of BL. Viral cooperation has been shown to be an important driver of oncogenesis. This study investigated the cooperation between HIV and EBV in the development of lymphoma.

METHODS: Luciferase reporter assays were used to assess the cooperation of HIV-1 Tat and LMP1 on the activity of AICDA regulatory sequences (AIDpR1, AIDpR2 and AIDpR4). Site-directed mutagenesis was used to interrogate the role of the Early growth 1 (Egr-1) transcription factor binding element in the Tat-mediated AIDpR1 response. Transfection and western blotting were employed to determine the effect of LMP1 expression on AID and c-MYC protein expression in the BL cell line, Ramos.

RESULTS: Tat and LMP1 cooperate to enhance the activity of AIDpR1 independently of Egr-1. Furthermore, LMP1 significantly enhanced the activity of AIDpR2 and AIDpR4 promoters. The AP-1 transcription factors (TFs) c-JUN and JUNB, known to mediate downstream LMP1 signalling, were shown to enhance AICDA promoter activity. Additionally, ectopic expression of LMP1 in Ramos increased AID and c-MYC protein expression.

CONCLUSIONS: HIV-1 Tat potentially cooperates with EBV to enhance the activity of the AIDCA promoters. This was independent of Egr-1, but may be mediated via the AP-1 TFs. Investigations to uncover this signalling axis is ongoing.

ABSTRACT WITHDRAWN

Mitochondrial DNA copy number determination in a South African cohort of Type 2 diabetes mellitus patients with target organ damage

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Presentation Slot: PS20

Abstract:

Introduction

Various aspects of mitochondrial function and dysfunction have been studied in human diseases, from rare inherited disorders to more common conditions such as neurodegenerative disease, cancer, and diabetes. However, limited reports exist regarding Type 2 diabetes mellitus (T2DM), and there is a need for comprehensive studies correlating variation in mitochondrial DNA copy number (mtDNA-CN) in blood with T2DM and its associated complications, also known as target organ damage (TOD).

The existing diagnostic methods and parameters used to assess T2DM and TOD are not sensitive enough to detect the occurrence of TOD, and by the time TOD is clinically detectable, significant damage has already occurred, and therapeutics are less effective. Hence, biomarkers, such as mtDNA-CN, that can assess disease severity are highly desirable.

Materials and Methods

Our study adopted a well-optimised ddPCR method to measure absolute levels of whole blood (WB) mtDNA-CN and peripheral blood mononuclear cells (PBMCs) mtDNA-CN in 34 T2DM patients with and without TOD.

Results and Discussion

Our findings showed no differences in mtDNA-CN distribution, whether measured in PBMCs or WB, based on the presence or absence of TOD. However, we observed a difference in PBMCs mtDNA-CN distribution according to the presence of dyslipidaemia as a co-morbidity of T2DM. Patients with dyslipidaemia had, on average, lower mtDNA-CN compared to patients without dyslipidaemia. This effect was more noticeable in patients without TOD.

In conclusion, when measuring mtDNA-CN in human blood as a biomarker, the specific sample type is an important factor to consider for the specific condition under assessment and the results obtained.

Development of recombinant immunotoxins targeting overexpressed tumour cell surface biomarkers Trop2 and Mesothelin for cervical cancer treatment

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Presentation Slot: PS21

Abstract:

INTRODUCTION

Cervical cancer is a devastating disease that poses health concerns in Sub-Saharan Africa. In South Africa, it is regarded as the second most common cause of cancer death with high incidence rates. The disease is attributed to the human papillomavirus (HPV). Screening for cervical cancer is deemed expensive, and the current conventional treatments, are not efficient and cause side effects. In this research we therefore propose to develop target specific therapeutics to reduce the burden. We seek to use recombinant immunotoxins that target specific cell surface biomarkers (TROP2/ Mesothelin) which are overexpressed in cervical cancer cells. The TROP2/Mesothelin single variable chain (scFv) will be fused to toxins deimmunised exotoxin A (DETA) or exotoxin A (ETA) which are known to be cytotoxic.

METHODS AND MATERIALS

The genes TROP2 (DETA/ETA) and mesothelin (DETA/ETA) were synthesized and cloned into pMT vector for expression. The recombinants were expressed in E. coli cells. The protein was extracted from the cells and purified using histidine affinity chromatography. The purified proteins were analysed and characterized and will be tested for their ability to bind to the over expressed TROP/Mesothelin receptors on positive cervical cancer cell lines. Moreover, their cytotoxicity effects will be assessed.

RESULTS

The recombinant immunotoxins were successfully cloned, expressed in bacteria and purified. The purified immunotoxins were analysed, characterized and quantified. Currently, the functionality of these immunotoxins is being tested.

DISCUSSION

Anti-TROP2/Mesothelin (scFv)-DETA/ETA can efficiently be expressed E. coli and purified. Future work will to ascertain the functionality of these recombinant immunotoxins is ongoing.

ABSTRACT WITHDRAWN

Characterisation of a public T cell clonotype enriched in M.tb infected individuals who do not progress to tuberculosis

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Presentation Slot: PS22

Abstract:
Introduction: T cells are known to play an important role in control of infection, but it is not known if specific T cell clones contribute to outcome of M. tuberculosis (M.tb) infection. We recently observed that frequencies of donor-unrestricted T cells (DURT) expressing a common CDR3a sequence, we termed "kiif.tb", was higher in healthy, M.tb-infected individuals who controlled infection, compared to individuals who progressed to tuberculosis (TB). Furthermore, frequencies of kiif.tb T cells were higher in healthy M.tb-infected individuals compared to persons diagnosed with TB. DURTs recognise antigens presented by molecules that, unlike MHC, are not restricted by genotype of the donor. As a result, a single vaccine immunogen might induce T cell responses in recipients of any genetic background.
Methods: Frequencies of T cells and kiif.tb T cells from healthy uninfected, healthy M.tb-infected, and TB patients were quantified by custom multiplex digital PCR (dPCR) assay. Identification and characterisation of kiif.tb T cells was performed using a flow cytometry-compatible RNA hybridization assay (PrimeFlowTM).
Results: Frequencies of kiif.tb T cells measured by dPCR correlated modestly with frequencies of kiif.tb measured by bulk TCR sequencing (assigned as gold standard). However, frequencies of kiif.tb T cells were not significantly higher in M.tb-infected, compared to healthy controls or active TB disease individuals, as hypothesized.
Discussion: Our results suggest that peripheral blood frequencies of kiif.tb T cells are not different between study groups. Alternatively, the dPCR assay might lack the sensitivity to detect differences in abundance of these T cells across the study groups.

Investigating the exacerbating effects of HIV-proteins on the BBB: an in vitro BBB model

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Presentation Slot: PS23

Abstract:

The blood-brain barrier (BBB) is vital for brain homeostasis and shielding the CNS from harmful substances. It comprises of specialized brain endothelial cells (BECs) with tight junctions that limit paracellular substance movement. Inflammation, like in HIV infection, can perturb BBB integrity in many ways, including BEC death, leading to heightened BBB permeability. This allows viral particle entry into the brain, leading to neurological complications like HIV-associated neurocognitive disorders.

Studying BBB integrity, viability, and toxicity in vitro unveils intricate mechanisms of HIV-induced BBB dysfunction. This experiment employed an optimized in vitro BBB model with immortalized mouse brain endothelial cells (bEnd5) cells and HIV-1 protein-expressing HL2/3 cells to emulate in vivo HIV-1 paracrine effects on an established BBB. Overall barrier integrity was assessed using transendothelial electrical resistance (TEER) which offers a quantitative means for determining BBB changes when BECs are exposed to HIV-1 proteins by measuring electrical resistance across the endothelial cell monolayer. bEnd5 cell viability and toxicity was assessed and a concentration-dependent response was noted for both parameters.

This experiment will add to the current body of knowledge concerning the mechanisms behind barrier disruption. It will establish a basis for potential therapeutic approaches focused on maintaining BBB integrity, alleviating neuroinflammation, and ultimately improving neurological outcomes for individuals impacted by HIV-associated inflammation.

HIV infection, secretor status and antiretroviral initiation timing influence HMO concentrations

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Presentation Slot: S1.5

Abstract:

Background: Human milk oligosaccharides (HMOs) are complex sugars in human milk that influence infant health and development. Previous studies report HMOs composition alterations in mothers living with HIV. This work investigates effect of HIV infection and ART duration in pregnancy on HMO composition in a cohort of mothers living with and without HIV. In addition, we investigate maternal factors such as secretor status, cytomegalovirus infection, and education.

Methods: As part of the Healthy Baby Study, mother-infant pairs were recruited from Khayelitsha in Cape Town, South Africa. Mothers living with HIV were subdivided based on ART initiation relative to conception date (pre/post conception ART initiation). Human milk samples were collected from mothers 2 to 4 weeks after birth. High performance liquid chromatography was used to quantify 19 HMOs, with HMO concentrations (ug/mL) and relative abundance reported. Statistical analyses was performed to identify HIV status and ART timing group differences as well as other significant maternal factors.

Results: We report results for 153 mother-infant pairs (93 mothers living with HIV). Non-secretor women living with HIV had higher median concentrations in LNFPIII independent of ART initiation in pregnancy. Among non-secretor women living with HIV, those who initiated ART before conception had higher median levels of FLNH whereas women who started ART post conception had higher median LNT concentrations. Secretor women living with HIV who initiated ART after conception had lower median concentrations of DFLNH.

Discussion: We find altered HMO concentrations in women living with HIV which depend on secretor status and ART initiation.

The anatomical variations of the recurrent laryngeal nerve and its relationship with the inferior thyroid artery.

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Presentation Slot: S3.7

Abstract:

Introduction: The recurrent laryngeal nerve is a branch of the vagus nerve. There is risk to the safety of surgical procedures in the neck region posed by limited knowledge regarding the anatomical variations of the recurrent laryngeal nerve (RLN) it's course and branching pattern. This study aims to assess the anatomical variations of the RLN within a South African cadaveric sample by looking at the potential anatomical variations of the RLN pertaining to its course, branching pattern and relationship to the inferior thyroid artery (ITA). Materials and Methods: This is a prospective, observational cross-section study with a descriptive analytic component. 48 formalin fixed bodies were dissected and examined. The branching pattern and relationship with the ITA was evaluated and documented bilaterally. Results and Discussion: The loop of the left RLN around the arch of the aorta was observed in only 41,7%. On the left, 47 RLN were showed 8.5% had no branching, 46.8% bifurcated, 23.4% trifurcated and 21,3% had multiple branches. On the right, 46 RLN were showed 6.5% had no branching, 47,8% bifurcated, 28,3% trifurcated and 17.4% had multiple branches. In relation to the ITA on the left the nerve was anterior in 19.1%, in between in 10.6% and posterior in 70.2%. On the right the nerve was anterior in 30.4%, in between in 34.8% and posterior in 34.8%. The information from this study is comparable to previous literature and reveals that the variability of the RLN is to be considered in surgeries of the neck to avoid damage.

Next generation version of Pseudomonas Exotoxin A based immunotoxins with reduced immunogenicity to target Triple Negative Breast Cancer (TNBC) cells

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Presentation Slot: PS24

Abstract:

TNBC, is one of the five intrinsic breast cancer categories, that lacks estrogen receptor (ER-), progesterone receptor (PR-) and human epidermal growth factor receptor 2 (HER2-) expression, resembling basal or myoepithelial cells. It affects younger patients, particularly premenopausal African women, often showing genomic instability and homologous recombination deficiency. This suggests potential sensitivity to DNA-damaging agents like platinum and DNA repair inhibitors, including poly(ADP-ribose) polymerase-1 inhibitors. TNBC, typically starts at a younger age, has a higher chance of recurrence, and a shorter survival time of around 10-13 months after spreading. TNBC is challenging to treat due to both clinical and molecular factors. It comprises about 15-20% of new breast cancer diagnoses and is more aggressive, with poorer prognosis and higher grade compared to other types, contributing to 5% of annual cancer-related deaths. Current treatments yield a progression-free survival of less than 3.5 months, highlighting the need for targeted therapies to enhance TNBC management.

The absent biomarkers in TNBC's responsiveness to chemotherapy has led us to emphasize personalized diagnostic and treatment strategies based on patients' genetic, phenotypic, and biomarker traits. Our study focuses on enhancing Pseudomonas Exotoxin A immunotoxins with reduced immunogenicity. These advanced immunotoxins offer specificity, effectively delivering the active toxin component to the cytosol through precise processing and trafficking mechanisms. The PE bacterial toxin alters eukaryotic elongation factor 2 (eEF2), a crucial protein synthesis element, leading to apoptosis and cell death. This innovation holds promise for tailored TNBC treatment, offering the potential for improved patient outcomes and quality of life.

Patient-specific Computational Study of Myocardial Mechanics for Improved Diagnosis of Peripartum Cardiomyopathy

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Presentation Slot: S2.5

Abstract:

Introduction

Peripartum cardiomyopathy (PPCM) occurs in women in the last month of pregnancy or several months after delivery. PPCM does not have a known cause, is diagnosed by exclusion, and is associated with high mortality (10-30%). The current study aimed to develop patient-specific cardiac models and investigate PPCM cardiac biomechanics characteristics suitable for improved diagnosis.

Materials and Methods

Three-dimensional biventricular cardiac geometries were reconstructed from cardiac MRI data for six PPCM patients (Simpleware ScanIP). Finite element models were developed (Abaqus) and coupled to a lumped-parameter representation of the circulatory system. Myofibre orientation was implemented using the rule-based method. Passive and active material properties were included in the constitutive formulation of the myocardium and optimized using the sequential least-squares method. Six cardiac cycles were computationally simulated, and myocardial strain and stress were recorded and assessed.

Results

In PPCM, the left-ventricular (LV) myofibre stress and strain were 30.4 ± 10.9 kPa and -6.7% ± 3.5% at end-systole and 6.1 ± 2.0 kPa and 4.4% ± 3.3% at end-diastole. The myofiber stress was two-fold higher in the LV than the right ventricle (RV) (14.6 ± 3.9 kPa, $p = 0.03$) at end-systole but similar to the RV at end-diastole (5.6 ± 1.8 kPa, $p = 1.55$).

Discussion

Myofibre strain and stress predicted for PPCM were similar to those previously reported for heart failure, and the current results have limited diagnostic value. However, the patient-specific PPCM cardiac models developed serve as a basis for further research towards improved diagnosis, prognosis and treatment of PPCM.

Investigating and evaluating the use of the ND4 marker for the effective taxonomic delineation of Lucilia sericata and Lucilia cuprina within the Western Cape of South Africa

Darshni Naiker[1], Kyle Kulenkampff, Laura Heathfield

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Presentation Slot: PS25

Abstract:

Investigating and evaluating the use of the ND4 marker for the effective taxonomic delineation of Lucilia sericata and Lucilia cuprina within the Western Cape of South Africa
Darshni Naiker, Kyle Kulenkampff, Laura Heathfield
Division of Forensic Medicine and Toxicology, Department of Pathology, Faculty of Health Sciences

Introduction:

Forensic entomology involves the use of insects to determine post-mortem interval, which relies on accurate species identification. DNA barcoding has been used for species identification, however, this methodology is limited for closely related species. The addition of barcodes is hypothesised to increase the discriminatory power of this technique. The aim of this study was to evaluate the use of the ND4 marker for the differentiation of Lucilia sericata and Lucilia cuprina.

Materials and methods:

Using available reference sequence data, primers for the ND4 region were designed, and a PCR assay was optimised using DNA from a subset of Lucilia specimens (n=12). The assay was applied to a larger sample set (n=59) from various localities in the Western Cape. Amplification was verified using gel electrophoresis and Sanger sequencing.

Results:

The optimised PCR used an annealing temperature (Ta) of 63.6°C and Sanger sequencing confirmed the correct ND4 barcode. However, when applied to the larger sample set, only 60% of the DNA samples were amplified. Lowering the Ta to 62.3°C improved results, with successful amplification for 79.41% of the samples. Further, optimisation did not improve results.

Discussion:

Overall, the primer set was ineffective in amplifying all the specimens, suggesting that there is novel sequence variation in the primer binding region of Lucilla species in the Western Cape. The primers would not be reliable for application in forensic casework and another region in ND4 should be considered. Whole genome sequencing of the Lucilla species found in the Western Cape is recommended to identify novel barcodes which are locally informative.

Development of an Ultraviolet Irradiation Device: Initial Bacterial Inactivation Profile

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Presentation Slot: S3.4

Abstract:

Introduction:

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. With globally increasing rates of antimicrobial resistance, the ability of physicians to treat the underlying infection that causes sepsis is diminishing. Before the discovery of antibiotics, ultraviolet irradiation of the blood was a treatment used to treat sepsis. Based on this therapy, this research project is developing a device to perform ultraviolet irradiation of blood products.

Materials and Methods:

A novel device using ultraviolet light of varying fluences was used to inactivate Staphylococcus aureus cells in phosphate-buffered saline (PBS). For this, PBS was spiked with a standardised number of bacterial cells and pumped through the UV-irradiation device. The log reduction factor of bacteria was determined by comparing colony plate counts of unexposed and UV-irradiated cells. Initial testing of reference strain S. aureus ATCC25923 was conducted by exposure to different UV fluences to generate an inactivation curve. The following experiments exposed drug-sensitive and drug-resistant clinical isolates to selected UV fluences. Data from triplicate, biological replicates were used to calculate the log reduction factor at different fluences.

Results and Discussion:

The bacterial inactivation curve observed was dose-dependent, with a mean log inactivation factor for ATCC25923 of 3.83 at the maximum tested UV fluence. In the clinical isolate experiments, comparable inactivation of the reference and clinical isolates was observed following exposure at a high UV fluence. These results are promising for the further development and application of the novel device developed in this study.

Investigating neural tube development using an in vitro stem cell-based model

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Presentation Slot: PS26

Abstract:

Introduction

The study of early embryogenesis has been transformed with the generation of in vitro stem cell-derived embryo models. Of particular interest are neural tube organoids (NTOs), which mimic neural tube (NT) formation, morphogenesis, and closure, enabling the study of human NT development.

The use of biochemical, e.g. Wnt agonists, and physical supplements, e.g. Matrigel, in culture have furthered NTO development, however the precise contributions of these various factors in driving morphogenesis have yet to be fully elucidated. Here, we aim to investigate the effects of two signalling molecules, Chiron and BMP4, on the development of NT cells in a mouse embryonic stem cell (mESCs) model.

Materials and Methods

500 mESCs/well were seeded in low-attachment 96-well plates and formed aggregates over 48 hours, with addition of 3µM Chiron or 1ng/mL BMP4 for 24 hours and were embedded in 5% Matrigel from 96 hours. Aggregates were collected at 96 and 144 hours for ICC and RT-qPCR, with growth analysed daily.

Results

Aggregates grown without signalling factors did not elongate or express Brachyury protein, a marker of the primitive streak, at 96 or 144 hours. Aggregates pulsed with Chiron or BMP4 elongated, with BMP4-pulsed aggregates showing higher expression of NT marker Sox2 compared to Chiron-pulsed aggregates.

Discussion

Adding Chiron and BMP4 with a Matrigel scaffold drives expression of NT markers in mESC aggregates. BMP4 addition results in higher expression of Sox2 than Chiron, indicating that it is able to drive NT development in vitro.

Digital PCR for the simultaneous enumeration and assessment of the replication potential of Mycobacterium tuberculosis.

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Presentation Slot: PS27

Abstract:

Molecular detection of Mycobacterium tuberculosis (Mtb) utilizing nucleic acid amplification has enabled rapid detection of the bacterium with a high degree of sensitivity and specificity. Expanding on that technology, we have developed a multiplexed droplet digital PCR-based (ddPCR) assay for simultaneous enumeration and viability assessment of Mtb in (extreme) paucibacillary clinical samples. Detection and enumeration of Mtb are enabled using two genomic DNA targets: the Mtb-specific region of difference 9 (RD9), which distinguishes Mtb from Mtb Complex organisms, and the multicopy insertion element, IS6110. In combination, we utilize determination of the ribosomal RNA synthesis (RS) ratio to obtain a transcriptomic measure of Mtb replicative potential. In proof-of-concept studies, we have quantified the number of Mtb bacilli and determined their replication potential during various growth phases in vitro, in tissue isolated from mice infected with Mtb, and in formalin-fixed paraffin embedded (FFPE) patient-derived samples. Moreover, we have demonstrated that the ability to detect Mtb using this multiplexed assay is independent of the nucleic acid isolation method employed. We are currently applying this approach in an ongoing investigation of post-mortem samples with the overarching objective of measuring viable Mtb numbers in different tissue microenvironments.

The prevalence and significance of MYD88^L265P and CD79^BY196 activating mutations in a South African cohort of DLBCL patients

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Presentation Slot: S3.6

Abstract:

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma (NHL) in adults, as well as in people living with the Human Immunodeficiency virus (HIV). Approximately 40% of patients do not respond favourably to standard therapy. This has been largely attributed to the high clinicopathological heterogeneity of the disease. Recent genetic studies have described a MYD88/CD79B-mutated (MCD) genetic subset of DLBCL with the co-occurrence of activating mutations in these two factors, characterised by poor prognosis. The aim of this project was to determine the prevalence of MYD88^L265P and CD79B^Y196 mutations in a local cohort of activated B-cell-like (ABC)-DLBCL patients.

Materials and Methods

Genomic DNA was isolated from Formalin-fixed paraffin-embedded (FFPE) tissues and the regions spanning the mutations of interest were amplified by PCR and sanger sequencing was used to identify the relevant mutations.

Results

The MYD88^L265P mutation was detected in 26% of the tumours, while mutations at CD79B^Y196 were observed in 12% of the tumours. Co-occurrence of both mutations were present in 3 HIV-negative patients. Kaplan-Meier survival analyses indicated worse overall survival for patients harbouring one or both mutations. Interestingly, this pathogenic effect of the CD79B mutations seem more severe and was comparable to the survival probability observed for HIV-positive patients.

Discussion

The findings of this pilot study confirm that MYD88^L265P and CD79B^Y196 mutations hold potential as independent prognostic markers for aggressive disease in DLBCL. Additionally, infection with HIV appears to compound the negative effect of harbouring the MYD88^L265P mutation on overall survival.

Elucidating interactions between the mycobacterial mutasome/SOS response and other DNA repair pathways

Atondaho Ramudzuli[1], Sophia Gessner, Mandy Mason, Digby Warner
[1]Department of Pathology, Faculty of Health Science, University of Cape Town, South Africa.

Presentation Slot: PS28

Abstract:

Introduction.

Mycobacterium tuberculosis employs different DNA repair mechanisms to maintain genome integrity and cell viability. This pathogen is also equipped with DNA damage tolerance mechanisms such as the SOS response that is induced under stress. DNA damage repair under conditions of genotoxic stress is known to be error prone potentially leading to induced mutagenesis. Characterizing factors involved in DNA metabolism under conditions of stress is critical for target-based drug design.

Materials and Methods.

A dual DnaN-mCherry::eGFP-ImuB fluorescent reporter was generated to screen for possible interactions between the SOS response and other components of DNA metabolism. In this background a pooled CRISPRi library targeting 142 genes involved in DNA metabolism, for partial knockdown, was generated. Nine colonies were selected for growth knockdown verification and characterisation of the co-localisation phenotype.

Results.

CRISPRi knockdown validation indicated that DnaN knockdown resulted in attenuated growth, DnaG knockdown only slightly attenuated growth, and knockdown of NrdH did not affect growth. Fluorescence microscopy revealed an increase in DnaN fluorescence intensity with knockdown of DnaG and NrdH cells under conditions of genotoxic stress. As expected no ImuB fluorescence signal was observed before DNA damage, in contrast to the increase in fluorescence intensity following exposure to DNA damage.

Discussion.

Since DnaG and NrdH are essential genes, the reduced sensitivity effect to ATC could be as a consequence of the intermediate knockdown sgRNA guides used to generate the library. Taken together, these observations support the power of using alternative tools such as microscopy to elucidate a phenotype in mycobacteria.

Evaluation of apoptotic impact of atorvastatin on peripheral blood mononuclear cells infected with Mycobacterium tuberculosis

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Presentation Slot: S3.3

Abstract:

Introduction: Tuberculosis remains a significant global health challenge, contributing substantially to morbidity and mortality rates. Atorvastatin is among the potential agents that exhibit anti-inflammatory and immunomodulatory attributes in addition to managing cholesterol synthesis. Apoptosis is one of the effector mechanisms that limit the intracellular growth of Mtb. Therefore, this study aimed to identify the mycobacterial killing capacity of atorvastatin-treated peripheral blood mononuclear cells (PBMCs) and investigate the apoptotic effects of atorvastatin following Mtb infection.

Materials and Methods: Blood samples from healthy donors were collected, and then PBMCs were isolated using Ficoll-Paque density media. Isolated PBMCs were pre-treated with different concentrations of atorvastatin (5-100 μM) and then infected with Mtb. Thereafter, the colony-forming units were calculated at 1 Day post-infection (dpi) and 3dpi. Additionally, various apoptotic assays were performed, such as TUNEL assay for DNA fragmentation, caspase-3 activity, and the expression of pro-apoptotic gene (Bax-1).

Results: Our investigation revealed that pre-treatment with atorvastatin led to a substantial reduction in mycobacterial load in infected PBMCs in a dose-dependent manner. Moreover, our findings unveiled a heightened apoptotic pathway induced by atorvastatin, as evidenced by increased fragmented DNA, escalated caspase-3 activity, and amplified expression of Bax-1 gene on Mtb-infected cells, setting them apart from untreated controls.

Discussion: The findings of the study are similar to Parihar et al. who confirmed the inhibitory effects of simvastatin on the growth of Mtb in infected macrophages. Also, it agreed with Guerra-De-Blas et al. in inducting apoptosis, which highlights its potential as a promising host-directed therapy against tuberculosis.

Repurposing drugs that target the oncogenic TBOX transcription factors TBX3 and TBX2 to treat pancreatic cancer

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Presentation Slot: S3.8

Abstract:

Introduction: In South Africa, 84% of patients diagnosed with pancreatic cancer (PC) die from the disease, therefore, new therapeutic agents are urgently needed. Repurposing commercially available non-cancer drugs that inhibit key drivers of PC may facilitate the rapid identification of cost-effective drugs. In PC the expression of TBX3 and TBX2 correlates with distant metastasis and poor patient survival. Here, we biologically validated TBX3 and TBX2 as therapeutic targets and investigated the effects of commercially available drugs, piroctone olamine (PO), and pyrvinium pamoate (PP) on the levels of TBX2 and TBX3 in PC.

Materials and Methods: Human PC cell lines (BxPC-3, CFPAC-1, PANC-1, and SW1990) were used in this study. Growth curve analyses, SA-β-Gal staining, clonogenic assays, scratch motility assays, transwell invasion assays, luciferase reporter assays, 3D spheroid formation and invasion assays, qRT-PCR, immunocytochemistry, and western blotting were used to validate TBX3 and TBX2 as therapeutic targets and to investigate the effects of PO and PP on TBX3 and TBX2 levels.

Results: TBX3 knockdown induced senescence, inhibited cell proliferation, 3D spheroid formation and growth, and epithelial-to-mesenchymal transition (EMT), but promoted cell migration and invasiveness of 2D and 3D PC cultures by transcriptionally repressing TBX2. Also, PO and PP significantly reduced the levels of TBX3 and TBX2 at their IC50 concentrations.

Discussion: TBX3 and TBX2 should be targeted concurrently for effective PC treatment and since PO and PP reduced the levels of TBX3 and TBX2, they have a great potential to be repurposed for the targeted treatment of PC.

Investigating cellular mechanics in endothelial cells infected with a recombinant Kaposi's sarcoma-associated herpesvirus

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Presentation Slot: PS29

Abstract:

Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV) targets endothelial cells and causes Kaposi's sarcoma (KS). Molecular mechanisms underlying KSHV-induced malignant transformation of endothelial cells are well described, but further elucidating KS's pathogenesis and progression remains crucial. KSHV infection alters the cytoskeletal and morphological properties of endothelial cells, which may impact intracellular mechanics. The current study aimed to quantify mechanical changes in human aortic (HuARLT2) and lymphatic (LEC) endothelial cells when infected with KSHV.

Materials and Methods

HuARLT2 and LEC were grown in 2D conditions and infected with a recombinant KSHV strain, rKSHV.219, by spinoculation. Infection was confirmed by GFP expression, and infected cells were maintained through puromycin selection to produce stable, latently-infected populations (HuARLT2-rKSHV and LEC-rKSHV). The mechanical properties of latently-infected and uninfected cells were probed with mitochondrial tracking microrheology, involving fluorescent labelling of mitochondria, timelapse confocal microscopy, and quantitative image analysis.

Results

HuARLT2-rKSHV and LEC-rKSHV displayed significantly lower stiffness than uninfected controls as quantified by the bulk mean squared displacement (MSD) and its power law exponent (α). HuARLT2-rKSHV and LEC-rKSHV exhibited an MSD increase of 41% and 40% and an α decrease of 24% and 37%, respectively, than their uninfected counterparts. The decreased stiffness in infected cells corresponded with observed changes in cell morphology.

Discussion

This study linked increased cell deformability to morphological changes previously reported with KSHV infection of endothelial cells. The observed stiffness change is a potential diagnostic and prognostic biomarker with clinical relevance to KS and other KSHV-associated diseases.

The development of recombinant immunotoxins for the treatment of triple negative breast cancer

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Presentation Slot: PS30

Abstract:

Introduction:

Among the subtypes of breast cancer, triple-negative breast cancer (TNBC) is one of the most aggressive and often has a poor prognosis. The currently available therapies such as chemotherapy and radiotherapy have been associated with many adverse reactions. Optimised treatment of TNBC needs to be further fine-tuned to combat this deadly disease in the most efficient and effective therapies possible. Immunotherapy considers the surface antigens, specifically expressed on TNBC cell surfaces and monoclonal antibody technology. However, to improve this form of treatment, more research needs to be directed toward improved recombinant immunodiagnostics and -therapeutics. Therefore, this study aims to generate Pseudomonas exotoxin A based recombinant immunotoxins (rITs) including next generation deimmunized variants to target overexpressed TNBC surface receptors. Preliminary in vitro studies will determine the overall protein efficacy to pave the way for an improved treatment to TNBC.

Methods and Methods:

The rITs were designed for and expressed in E.coli cells and then purified using IMAC. Proteins were confirmed through SDS-PAGE and Western Blot protein analysis. The rITs were fluorescently labelled and antigen-antibody binding were evaluated in vitro using confocal imaging. rIT-mediated cytotoxicity were also evaluated, in vitro, using XTT-based cell viability assays.

Results:

rITs targeting four different antigens, were bacterially expressed; selective binding to antigen-positive TNBC cell lines was conducted prior to confirmation of dose dependent toxicities.

Discussion / Conclusion:

Protein characterisation, confocal imaging and XTT-based cytotoxicity assay data will be discussed and findings compared to other previously produced rITs.

Regulators of neuroimmune function in the manifestation of central nervous system tuberculosis in the absence of microglia.

Limpho Thipane[1], Avril Walters, Sohair Geyer Benjamin, Nai-Jen Hsu and Muazzam Jacobs

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Presentation Slot: PS31

Abstract:

Introduction

Tuberculosis (TB) is an ancient, chronic disease triggered by the bacillus Mycobacterium tuberculosis (M. tuberculosis). Among the most devastating forms of extrapulmonary TB, central nervous system tuberculosis (CNS-TB) is strongly associated with elevated mortality and morbidity. Several studies have reported that resident CNS cells, particularly microglial cells form part of the initial line of defense and are regulators of the innate host immune response in CNS-TB. The colony-stimulating factor 1 factor (CSF-1R) receptor is a crucial regulator of microglia.

Materials and methods

C57BL/6 female adult mice were orally administered with the AIN-76A (normal diet) and PLX5622. After set time points, brains from both groups were processed and analyzed by immunohistochemistry and flow cytometry to determine microglial depletion. Neuro-2A, a mouse neural crest-derived cell line was infected with Mycobacterium bovis BCG-GFP at a multiplicity of infection of 30:1, stained and processed for immunocytochemistry to determine the percentage and illustrate the bacilli internalization.

Results

Flow cytometry analysis showed a decrease of 87% of Iba-1+ microglial population in the PLX5622 treated group compared to the control group. There were no significant differences in percentages of neurons and astrocytes between PLX5622 treated and untreated group. Flow cytometry analysis showed infected neuro-2A had >15% of internalization compared to the uninfected group.

Discussion

These findings establish that microglia cells are dependent on CSF-1R signaling and the microglial depletion model can be used to study the function of microglia in CNS-TB. In the absence of microglia, both astrocytes and neurons can potentially contribute to host defense during infection.

Systematic KSHV genome analysis from a South African HIV/KSHV-positive patient cohort

Melissa Thomas[1], Vickie Marshall, Charles Goodman, Elena Cornejo-Castro, Melissa Blumenthal, Humaira Lambarey, Denise Whitby, Georgia Schafer

[1]Department of Integrative Biomedical Sciences, Faculty of Health Science, University of Cape Town, South Africa.

Presentation Slot: S1.2

Abstract:

Kaposi sarcoma herpesvirus is an oncogenic virus and the etiological agent of Kaposi's sarcoma, the most common AIDS-associated cancer. KSHV infection occurs primarily in Sub-Saharan Africa where it is also associated with other malignancies such as MCD, PEL and KICS, which also occur primarily with HIV coinfection. It is assumed that most individuals acquire KSHV infection during childhood where it remains undetected until later in life when the burden of HIV takes its effect on the immune system.There are 6 predominant KSHV subtypes world-wide namely A, B, C, D, E and F which are determined by the highly variable K1 region of the KSHV genome. Sequencing and characterizing the circulating subtypes in a specific geographical region aid in tracking evolutionary changes as well as malignant outcomes associated with different subtypes. Hence, the aim of this study was to determine the circulating KSHV subtypes in the Western Cape region of South Africa. A total of 58 DNA samples isolated from peripheral blood of HIV/KSHV co-infected patients were selected according to KSHV viral load and volume. Of this, 20 were successfully Sanger sequenced to determine K1 subtype. Additionally, 9 samples met the criteria for whole-genome sequencing. This study produced 29 K1 sequences of which 26 were of the A subtype, specifically A5, and the remaining 3 were of the B subtype, namely B2. These results are confirmed by previous studies from the same region where a trend of high A5 and B subtypes in AIDS-KS patients was reported. ORF-K15 is another variable and lytic gene associated with KSHV subtyping (alleles M, N and P). This gene has shown to induce the expression of multiple cytokines and activating several pathways linked to tumour pathogenesis. For the K15 allele, the results include 2 P-, 3 M- and 4 N-subtypes. These results point towards an interesting distribution of subtypes contributing to previous reports of K15 P and M subtypes being the most prevalent in Africa. There are limited whole-genome sequences available for South Africa, therefore these sequences provide a significant contribution to the pool of sequences available for this region.

Eyes never lie: utilising quantitative measures of eye colour to estimate the age of Chrysomya chloropyga pupae

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[1]Department of Pathology, Faculty of Health Science, University of Cape Town, South Africa.

Presentation Slot: S2.7

Abstract:

Introduction

Age assessment of blowfly pupae collected from death scenes can be used to estimate the post-mortem interval (PMI). Eye colour changes gradually over the pupal period, but description in nominal terms is subjective. Quantitative assessment by measuring the contrast between eye colour and a standard grey background can allow for more accurate estimates of pupal age. This study investigated the possibility of quantitative assessment of eye colour of the blowfly Chrysomya chloropyga as a tool for age estimation.

Materials and methods

Pupae reared under controlled conditions were sampled and observed in 6-hourly age intervals for the duration of the puparial period (n=15/age interval). After removing the pupal casing, pupae were observed under a stereomicroscope. Images of the eye and corresponding grey background of were collected and imported into Adobe Photoshop 2022. The mean RGB intensity of the grey background was manually adjusted to 150. This generated an exposure-change factor which was applied to the corresponding eye image. The mean RGB intensity of the eye was subtracted from that of the background, providing an eye-background contrast value.

Results

Visual assessment indicated a gradual change in eye colour over the last 50% of the puparial period. This was quantitatively confirmed as eye-background contrast increased as pupal age progressed.

Discussion

This study measured the contrast between RGB intensity of pupal eyes and a corresponding grey background, allowing for objective quantitative analysis of changes in eye colour related to pupal age. This method can be utilised for objective and quantitative assessment of pupal age.

Deciphering the dynamic gene regulation driving differential gene expression in the maturing human brain.

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Presentation Slot: S2.2

Abstract:

Introduction: During brain maturation, spatiotemporal gene expression is tightly regulated by complex gene regulatory networks (GRNs), which control cell development and function. These networks describe the interaction between transcription factors and cis regulatory elements of their target genes (i.e. promoters and enhancers). This study aims to identify enhancers that are differentially regulated between paediatric and adult brain samples to contribute to our understanding of the GRN that guides human brain maturation.

Methods: Enhancers are found in non-coding regions of the genome and when active are found in open chromatin regions. Assays for transposase-accessible chromatin (ATAC-seq) is used to identify open chromatin regions. Bulk ATAC-seq libraries were generated from eight paediatric and two adult fresh and frozen temporal lobe brain tissue samples. Sequenced ATAC-seq libraries were processed using two published bioinformatics pipelines involving pre-alignment quality control, alignment to the human genome, post-alignment quality control, peak calling, peak annotation and differential accessibility analysis.

Results: A consensus peak set of 69152 accessible regions, including putative enhancer and promoter regions, was generated. Preliminary differential accessibility analysis between adult and paediatric samples over these consensus peaks revealed several differentially accessible regions.

Discussion: The differentially accessible peaks from the two pipelines will be compared to identify any similarities. Peaks that have been identified by both methods will provide confidence in the data and assist in the shortlisting of putative enhancer regions that guide brain maturation. These enhancer regions will be validated in enhancer-reporter assays in organotypic brain tissue slices.

Mortality due to gendered violence and life stage vulnerability in South Africa.

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Presentation Slot: PS32

Abstract:

Introduction:

Injury mortality is a significant public health concern in South Africa, with previous research providing a snapshot of the gender and age mortality patterns. However, the role of sociocultural, economic and structural forces in shaping these violent and accidental deaths is relatively unexplored. To understand the ways that gender and life stage are conceptualised and discussed in forensic-based research, and whether these patterns are indicative of disparities and inequalities, a thematic analysis approach was employed.

Materials & Methods and Results:

This study utilised a mixed methods approach. In quantitative analyses, men faced a fourfold higher homicidal rate than women with gendered violence being identified as a pivotal cause of injury mortality among both men and women. This finding was in keeping with the global epidemic of violence against women and children, however, relatively unexplored in understanding violence against men and boys.

Additionally, the highest death rate occurred during early adulthood (20-34 years). While young adults appeared to be most at risk, unique challenges that amplify the risk of injury mortality within life stages were identified as contributors to heightened risk of violence.

Discussion:

This research uncovered the complex, interconnected network and nuanced dynamics that underpin injury susceptibility and risk across different genders and ages. These themes illustrated how power imbalances, social norms, inequities, and depictions of masculinity contribute to gendered violence. Whereas developmental processes and pressures perpetuate violence during the life stages. The findings presented may be used to inform interventions that can help reduce the disproportionately high injury mortality rates in South Africa.

Aqueous extracts of Dodonaea viscosa induce potent and selective cytotoxicity in DLBCL cells.

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Presentation Slot: S3.2

Abstract:

Introduction: Non-Hodgkin lymphoma (NHL) affects 1.5 million people worldwide and includes a wide range of lymphoid neoplasms. Among these, diffuse large B cell lymphoma (DLBCL), a highly aggressive B-cell lymphoma, is the most common type of NHL. In Southern Africa, DLBCL is the most prevalent NHL among HIV infected individuals. The disease displays vast heterogeneity at the molecular and genetic levels, as well as in treatment response and clinical outcome. Dodonaea viscosa extract (DVE), derived from a plant commonly used by traditional healers in the Western Cape, was examined for its cytotoxic activity against DLBCL cells.

Methods: The IC50 of DVE against two DLBCL cell lines (HBL-1 and SU-DHL-4) was determined, relative to a non-cancerous lymphoblastoid cell line (PB-LCL-B95-8H) using viability assays. Thereafter, the mechanism of DVE-induced cytotoxicity was investigated using microscopy, Annexin V incorporation and caspase activity assays, cell cycle analysis, colony formation in semi-solid media, proliferation-tracking assay and expression of apoptotic markers using western blotting.

Results: A highly favourable selectivity index of 3 was determined for DVE against DLBCL cells, which was reflected in colony forming assays, demonstrating significant and preferential inhibitory effects of DVE against lymphoma cells, compared to LCLs. Furthermore, widespread apoptosis was observed through Annexin V incorporation, of 10-Fold and 2-Fold increases for SU-DHL-4 and HBL-1 respectively, upon DVE-treatment, which was corroborated by western blotting and morphological analyses.

Discussion: These results revealed potent and selective cytotoxicity against DLBCL cells by the Dodonaea viscosa plant extract, and therefore a promising source of novel anticancer bio-compound.