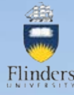
Flinders Centre
for Innovation
in Cancer



Flinders Centre for Innovation in Cancer 2018 Research Day

Student Poster Abstracts

Tuesday, September 4th, 2018

FCIC Function Room
Ground floor, FCIC building

Long non-coding RNA-protein interactions and butyrate sensitization of colorectal cancer cells

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Colorectal cancer (CRC) is the second most common cause of Australian cancer related deaths. The development of CRC is associated with epigenetic alterations including altered histone acetylation patterns and dysregulated long non-coding RNA (lncRNA) expression. Butyrate, a short-chain fatty acid, produced from the fermentation of dietary fibre in our gut, has been shown to alter CRC cell behaviour through epigenetic mechanisms. Butyrate can alter CRC gene expression, including lncRNA expression, via histone deacetylase inhibition activity, resulting in decreased proliferation and increased apoptosis. lncRNAs regulate gene expression through various mechanisms including epigenetic modifications, lncRNA-miRNA, lncRNA-mRNA, lncRNA-protein interactions and their ability to produce regulatory ncRNAs, such as miRNAs. lncRNAs have been shown to regulate cell growth and apoptotic pathways in CRC. The effect of exposing CRC cells to the anti-tumorigenic molecule, butyrate, in combination with lncRNA knockdown has yet to be investigated. High throughput functional screens were used to systematically identify oncogenic lncRNAs, which when knocked down resulted in the sensitization of CRC cells to butyrate (enhanced anti-proliferative and pro-apoptotic effects). Knockdown of some lncRNAs resulted in enhanced apoptosis in the presence of butyrate. Pathway and network analyses assisted in identification of predicted key lncRNA-protein interactions involved in apoptosis. Further investigation of lncRNA knockdown and their protein interactors in the context of butyrate is required. Identification of oncogenic lncRNAs, and protein interactors, with the ability to sensitise CRC cells to butyrate when suppressed, may reveal the potential chemo-preventive or therapeutic value of these biological molecules.

Expression analysis of sphingolipid homeostatic genes in chronic lymphocytic leukaemia lymphocytes: Can we find new therapeutic targets?

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Background: Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia in Australia, characterised by the clonal proliferation of CD5+/CD19+ B-lymphocytes. The disease is clinically heterogeneous, difficult to prognosticate, and is currently incurable; hence the need to identify novel prognostic markers and therapeutic targets. Our lipidomic analysis on CLL and control B-lymphocytes found the ratio of glucosylceramide (anti-apoptotic sphingolipid) to ceramide (pro-apoptotic sphingolipid) increased by 360%. This ratio is maintained between patients with aggressive disease compared to indolent disease. The balance of ceramide and glucosylceramide is essential for normal cellular function and lifespan. We hypothesise an imbalance in these sphingolipids may promote anti-apoptotic/chemoresistant characteristics in CLL B-lymphocytes.

Aims: 1) To optimise primers for the sphingolipid pathway genes of interest for quantitative PCR (qPCR).

2) Compare transcript levels of sphingolipid pathway genes in cryopreserved against fresh samples from the same patient in order to determine if cryopreservation affects gene expression and therefore whether cryopreserved samples are appropriate to use for expression analysis.

3) To observe changes in sphingolipid homeostatic genes at the mRNA level by qPCR in normal and CLL B-lymphocytes, and also in CLL B-lymphocytes from patients with aggressive and indolent disease.

Methods: Primers were designed and optimised for use on the ViiA7 (Life Technologies) qPCR machine for the following genes: glucosylceramide synthase (*UGCG*), β-glucocerebrosidase (*GBA*), lactosylceramide synthase, (*B4GALT6*), β-galactosidase (*GLB1*), α-galactosiadase A (*GLA*), and P-glycoprotein (*ABCB1*). Reference genes: β-2-microglobulin (*B2M*), glucuronidase-β (*GUSB*) and hypoxanthine phosphoribosyltransferase 1 (*HPRT1*). B-lymphocytes were isolated from control and CLL individuals, RNA was extracted for qPCR. Data was analysed using Quant-studio real-time PCR software.

Results: Primers were optimised for qPCR and confirmed to produce a single product of the correct size. It was found that cryopreservation does not significantly affect gene expression when compared to freshly preserved CLL cell samples. Expression of *UGCG* was found to be increased (5.2-fold, $p < 0.02$) in CLL compared to control B-lymphocytes.

Conclusion: Cryopreserved samples are appropriate for use in gene expression analysis of the sphingolipid pathway. Gene expression analysis supports the protein and lipidomic data indicating a shift towards anti-apoptotic/chemoresistant pathways in CLL.

Understanding lipid metabolism in Chronic Lymphocytic Leukaemia

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Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the Western world and primarily affects the elderly. CLL is characterised by the accumulation of mature B-lymphocytes in the blood and lymphoid organs. Patients often relapse from standard therapy, however the underlying cause is often unknown. Although many prognostic markers in CLL exist, one or a combination of these are unable to accurately predict the disease course for an individual.
Previous data from our laboratory demonstrates that circulating CLL B-lymphocytes endogenously synthesise lipids in the periphery, and reveals that CLL B-lymphocytes in the lymph node lymphoproliferative compartment have increased expression of proteins involved in β-oxidation. Results also showed a large increase in the levels of phospholipids associated with lipid droplets in CLL B-lymphocytes compared to normal B-lymphocytes.
The overarching hypothesis of this study is that circulating CLL B-lymphocytes uptake fatty acids (FA) for storage as lipid droplets (LD) for subsequent β -oxidation in the lymphoproliferative compartments in order to meet energy demands; and that CLL B-lymphocyte survival is dependent on key players in these pathways. Therefore, this study aims to identify important players in lipid metabolism by assessing gene expression of a panel of genes involved in lipid metabolism pathways in CLL B-lymphocytes.
B-lymphocytes were isolated from whole blood from control and CLL individuals, and RNA was extracted for use in gene expression analysis. The expression of a panel of 88 genes involved in lipid metabolism were assessed in control and CLL B-lymphocyte samples using qPCR.
Thirteen genes were found to be differentially expressed in CLL B-lymphocytes compared to normal B-lymphocytes. Differentially expressed genes were identified to be involved in lipolysis, lipogenesis, lipid transport, LDs, and cellular proliferation. Gene expression analysis suggests decreased lipolysis and lipogenesis in CLL B-lymphocytes compared to healthy B-lymphocytes. Analysis also suggests an increase in FA uptake and the formation of LDs in CLL B-lymphocytes compared to healthy B-lymphocytes.
This study provides a basis for further investigation into the 13 genes found to be differentially expressed in CLL B-lymphocytes compared to normal B-lymphocytes, and has the potential to identify novel therapeutic targets for CLL.

Putative driver mutations associated with trisomy 12 chronic lymphocytic leukaemia

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Trisomy 12 is a recurrent aneuploidy in chronic lymphocytic leukaemia (CLL) that confers a unique phenotype with increased expression of the integrin CD49d[1-3]. It is thought to be a founder event in CLL[4], but despite this, the molecular pathogenesis of trisomy 12 CLL is poorly understood.

Aim

To identify driver mutations in trisomy 12 CLL

Method

Two separate leukaemic clones were separated by flow cytometry based on bimodal expression of CD49d from an individual case of trisomy 12 CLL. Each leukaemic clone was subjected to immunoglobulin heavy chain variable region (*IGHV*) gene sequencing and whole exome sequencing (WES). CD5-positive T-cells from the same individual were also analysed.

Result

Trisomy 12 was present in high frequency (89.9%) in the CD49d+ fraction but was absent in the other fractions. The CD49d+ and CD49d- fractions harboured different *IGHV* rearrangements confirming two unique leukaemic clones. WES identified 8 mutations common to both clones (absent in T-cells) and 55 mutations unique to the CD49d+ clone alone. Two mutations were thought to be potentially pathogenic based on variant allele frequency and predicted function (*BCL11B* and *KMT2D*).

Conclusion

This highly unusual case provides insights into the evolution of CLL whose cell of origin is still debated. Importantly, the common mutations suggest that each clone arose from a single cell of origin and two potential trisomy 12 drivers were identified. Work is underway to interrogate additional samples and the functional impact of the mutations.

Harnessing miRNAs to enhance the anti-cancer properties of metformin in colorectal cancer

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Colorectal cancer (CRC) is the third most prevalent cancer in the world. The diabetes medication, metformin, is linked to cancer prevention and may selectively repress cancer proliferation. MicroRNAs are small non-coding RNAs involved in most cellular processes. Although metabolic consequences of metformin treatment have been investigated, detailed analysis of the resultant changes in gene expression is still required. Also, the effect of metformin treatment in combination with anti-cancer miRNAs has yet to be explored.
Full transcriptome and small RNA next generation sequencing were performed for CRC cells treated with metformin. Following differential expression, functional enrichment and network analyses, CRC cells were transfected with miRNA mimics to explore the anti-cancer effect of differentially expressed (DE) miRNAs. In addition, unbiased high throughput functional screens of a synthetic miRNA library, in combination with metformin treatment, were used to identify additional miRNAs that impact the metformin response.
Potential protein-protein interactions, within specific biological pathways that are affected by metformin treatment, were extrapolated from DE mRNAs and miRNAs and used to build system networks. Also, metformin treatment resulted in downregulation of some pro-proliferative and upregulation of some anti-proliferative miRNAs. Furthermore, miRNAs that sensitize CRC cells to the anti-cancer effect of metformin were experimentally validated. Identification of miRNAs that sensitise CRC cells to metformin, and their potential transcript targets, are early steps in the design of innovative therapeutic strategies.

Exosomes: A promising biomarker for precision medicine

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Aims

Exosomes are a subset of extracellular vesicles that circulate extensively throughout the body's biofluids which contain genomic information[1, 2]. Exosomes are proposed to have roles in cell-signalling as well as contain disease specific information[3]. Thus it is hypothesised that exosomes can be used as a biomarker for drug metabolising enzyme expression. This study aims to detect the change of exosomal mRNA after perturbing human hepatic cell lines exposed to enzyme-inducing agents and to quantify the activity of CYP3A4 within human plasma exosomes.

Methods

HepaRG, a human hepatic cell line derived from a hepatocarcinoma patient, were cultured until reaching differentiation state. The cells without intervention was cultured in exosome-depleted media whereas the intervention group was exposed to rifampicin for 72 hours. Real-time polymerase chain reactions were performed on harvested cells and media using SYBR green for quantification of CYP3A4 and GAPDH, both in cells and exosomes.

Exosomes in plasma were isolated from healthy volunteers. Nanoparticle tracking analyses were performed for exosomes quantification. Substrate-depletion assays were conducted using midazolam as a probe substrate for CYP3A4. The assays were performed using liquid-chromatography attached with mass spectrometry. Human liver microsomes were employed as a positive control. Midazolam 1-hydroxylation was measured in order to determine the magnitude of CYP3A4 activity.

Results

The mRNA fold change in both exosomes and HepaRG cells correlated with the change in its original cell experiments with 3 and 12 fold change of CYP3A4 mRNA respectively.

For the human plasma exosomes, midazolam 1-hydroxylation was detected after substrate-depletion with midazolam, suggesting that CYP3A4 was responsible for metabolite formation.

Conclusion

Using exosomes to assess the change of internal homeostasis is feasible. The benefit of using exosomes are not only minimising the invasiveness of intense sampling methods but also allows the possibility for early detection and a promising biomarker to investigate variability in drug-metabolising enzymes, providing a new paradigm for cancer research or rare diseases.

The development of an English as a second language cancer prevention curriculum for new immigrants to Australia: A translational research approach

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Background: Australian immigration patterns are changing, and the country is becoming increasingly multicultural. This pattern brings health implications. Disparities are noted in cancer incidence and mortality, as well as in uptake of cancer prevention activities such as screening. While many immigrants arrive to Australia with knowledge and skills to manage their health, typically little is known about Australia's cancer risks, optimal cancer prevention strategies or available cancer screening resources. Others may have less proficient English language skills to be able to obtain, understand and use available resources. Approaches that blend health literacy into existing migrant adult English as a second language (ESL) education show promise as a way to improve health literacy in new immigrants while improving language proficiency. Adopting a translational research approach may help in the development and evaluation of a curriculum that could reach across multiple cultures and geographic locations within Australia. **Method:** A cancer prevention awareness curriculum was developed in two stages: (1) using a participatory action research approach, focus groups and interviews were held with key stakeholders: ESL teachers and students. The RE-AIM translation evaluation framework (evaluating barriers and facilitators to intervention *reach*, *efficacy*, *adoption*, *implementation* and *maintenance*) underpinned interview questions and analysis of transcripts. (2) Based on the results of this study, a draft curriculum was produced and returned to the stakeholders for feedback via a quantitative survey to establish the potential reach of the curriculum's components to different genders, ages and cultures. **Results:** Data collection for stage 2 feedback is underway in Adelaide, South Australia (N=25 to date). **Conclusions:** Results from stage 2 will help to shape the final form of the curriculum to be trialled in 2019 for efficacy to improve students' health literacy and knowledge regarding cancer risks and prevention strategies, primary prevention intentions, behaviours and English language skills. The trial will also examine the degree of the curriculum's adoption by teaching staff, implementation into existing programming and maintenance of primary outcomes over time. **Clinical implications:** If found to be efficacious, this approach could be a feasible alternative to traditional health messaging for migrants to Australia.

Towards better diagnostic panels in the Immunohistochemical analysis of Lung cancer

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Lung cancer is a major health problem worldwide with very low 5-year relative survival rates (14%). Approximately 85% of lung cancers are Non Small Cell Carcinomas (NSCLC) that include the subtypes Adenocarcinomas (ADC) and Squamous Cell Carcinomas (SCC).

Often the diagnostic sample from the tumor is a biopsy with minimal tumor tissue. It is thus imperative to conserve tissue for molecular testing. i.e. EGFR, ALK, ROS1, BRAFV600E mutations and PDL-1 that would decide the chemotherapeutic or targeted therapy that patients may receive. Small tumor sample size, poorly differentiated tumors and colocalisation of squamous and adenocarcinoma markers on the same tumor cells are factors that make diagnosis difficult. Thus a limited panel of antibodies with higher diagnostic precision is preferred.

The most commonly used panel consists of TTF-1 that labels ADC and p40 that labels SCC. These markers are known to be highly sensitive and specific. However, results between different TTF-1 clones used have varying sensitivities, making diagnosis potentially difficult. We therefore undertook to examine a retrospective cohort of 200 NSCLC previously diagnosed cases at Flinders Medical Centre to include 116 SCC and 84 ADC specimens.

We found that the best 4 marker IHC panel was TTF-1 (clone SP141), NapsinA, p40 and CK5/6 with a sensitivity of 80% and specificity of 83% in differentiating ADC and SCC correctly. When other TTF-1 clones were used the sensitivity was lower. The panel that used p63 instead of p40 however had the highest specificity. A two marker panel with TTF-1 and p40 and CK5/6 revealed that both the panels had nearly the same sensitives. The panel of TTF-1 (clone SP141) and CK5/6 had 82% sensitivity and 84% specificity while the combination of TTF-1(clone SP141) and p40 had 81% sensitivity and 86 specificity. The highest specificity was seen with the combination of TTF-1(clone SP141) and p63 (87%). Despite the different clones of TTF-1 varying in performance, the panel with the highest specificity was one with p63. Our study reveals the utility of including the TTF-1 clone SP141 with much higher sensitivity than either clones SPT24 or 8G7 when used in a 2 or 4 marker panel for the immunohistochemical diagnosis of NSCLC.

Analysis of hereditary and putative breast cancer susceptibility genes in *BRCA1* and *BRCA2* mutation negative individuals.

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Breast cancer is the most common cancer affecting Australian women, with many individuals having a strong family history of the disease. Whilst inherited mutations in BRCA1, BRCA2, and additional susceptibility genes account for ~30% of familial breast cancer cases, the underlying cause in the remaining 70% is unknown, suggesting that additional breast cancer susceptibility genes exist. We hypothesised that mutations within genes that play a role in the DNA damage repair and checkpoint control pathways may be involved in predisposing families to inherited breast cancer.

In order to test our hypothesis, Ion Torrent Massively Parallel Sequencing and a custom targeted panel were used to sequence 51 genes of interest in 132 BRCA1/2 mutation-negative individuals with familial breast cancer. The gene panel consisted of 19 known breast cancer susceptibility genes (diagnostic genes) and 32 genes which play integral roles in the aforementioned pathways and therefore are potentially involved in the development of breast cancer (discovery genes).

Sequencing analysis identified ~120 variants in each sample, with variants present in <5% of the population analysed further. Of the 173 variants which were predicted to alter gene transcription or translation, 71 variants (42 diagnostic; 29 discovery) were identified as being potentially pathogenic. A pathogenic truncation mutation was identified in *PALB2* in 2 individuals. CRISPR/Cas9 was used to functionally validate a *UIMC1* polymorphism identified in 2 patients.

This research has the potential to provide much needed diagnostic information for the identification of mutations resulting in familial breast cancer, and to identify novel breast cancer genes.

Characterisation of Regulated Circular RNAs Across Human Embryonic Stem Cell Differentiation

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Metastasis, which is the movement of cancer cells around the body, is responsible for over 90% of cancer deaths. Despite this, the molecular changes occurring at the molecular level in the critical early stages of metastasis remain poorly characterised. To investigate more deeply these changes at the transcriptional level, I will exploit a human cellular model of a key process of metastasis, called epithelial-mesenchymal transition (EMT). EMT is the conversion of stationary cells into more motile cells and human embryonic stem cells, which are able to differentiate into all cell types, undergoes EMT during differentiation, therefore I will be utilising EMT and embryonic stem cell differentiation as models for cancer metastasis.
Circular RNAs (circRNAs) are a highly abundant class of RNA ubiquitous among eukaryotes usually located within protein coding genes. A small repertoire of functions have been recently reported for some circular RNAs (i.e. microRNA sponge, protein coding, protein binding), however the vast proportion of circRNAs remain functionally enigmatic. High-throughput RNA-sequencing of human stem cell differentiation into 5 different lineages has identified circRNAs that are commonly regulated during each EMT and also lineage-specific circRNA candidates. Furthermore, unique characteristics of individual circRNAs, including intron retention, along with the capacity to interact with RNA and proteins provides functional capabilities for gene regulation. We will manipulate the expression of these circRNAs through overexpression and knockdown experiments to study their effect on EMT. Furthermore, targeted capture of specific circRNAs will permit identification of interacting partners towards identifying what role these circRNAs could play in EMT, stem cell differentiation and cancer metastasis.

The development and validation of a questionnaire to assess the illness cognitions of young people who have a parent with cancer

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More than half of young people aged 12-24 years who have a parent with cancer experience clinically-elevated levels of depression and anxiety. Previous research indicates that the perceptions that young people have about their parent's chronic illness influences their ability to cope with, and adjust to, the illness. However, this relationship remains largely unexplored in the context of parental cancer. Furthermore, there are currently no psychometrically validated instruments available to measure illness perceptions in young people who have a parent with cancer. We are undertaking research to fill this gap, with a series of studies that aim to develop and validate an instrument to assess illness perceptions in this cohort.
We recently conducted semi-structured interviews with young people to explore their beliefs about, and perceptions of, their parent's cancer. Eleven young people aged 15-24 years participated in interviews. Of those, two participants had both parents who had been diagnosed with cancer, four participants were bereaved, and four participants had a parent with advanced or metastatic cancer. Interview transcripts were analysed using deductive thematic analysis techniques and the Common-Sense Model of Self-Regulation as a framework. Major themes aligned with the dimensions of the model, with young people describing their parent's cancer with reference to cognitive (identity, coherence, consequences, control, timeline, and cause) and emotional representations. Young people discussed the negative impact of their parent's cancer, but also highlighted positive consequences such as strengthening relationships with friends and family. The findings have been used to adapt the existing Perceptions of Parental Illness Questionnaire, which was originally developed for young people who have a parent with multiple sclerosis. We are currently conducting structured cognitive interviews with young people who have a parent with cancer to obtain expert feedback about the adapted measure. The findings will be used to refine the measure. Future research will be conducted to further evaluate the psychometric properties of the refined measure and examine the relationships between specific types of perceptions and young people's psychological outcomes. Together, the outcomes from these studies will improve our understanding of the mechanisms by which parental cancer affects young people; enhance the ability to identify young people at risk of poor mental health outcomes; and inform the development of effective strategies to improve outcomes and promote positive psychological growth in young people who have a parent with cancer.