RF1 Assessment of Prostate Tumor Heterogeneity Using Machine Learning: An Emerging Imaging Tool for Clinical Practice

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Background

Multiparametric MRI (mp-MRI) combines anatomical with different functional techniques to achieve improved accuracy or tissue characterization and is routinely applied in the study of prostate cancer. The increased number of images generated during mp-MRI increases image interpretation time and interpretation difficulty. Furthermore, tumor heterogeneity is an important factor, which may be reflected in mp-MRI. Machine learning has the potential to improve the efficiency and consistency of prostate mp-MRI interpretation and aid the accuracy of prostate lesion detection and assessment and provide a consistent response to treatment evaluation.

Aims

The purpose of this study was to develop a binary and probabilistic machine learning model for peripheral zone (PZ) prostate cancer using mp-MRI including T2WI, diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI).

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Methods

Twelve high-grade prostate cancer patients signed written consent prior to participating in this study. Cancer and healthy region of interests (ROI)s were outlined in the peripheral zone (PZ) by a radiologist. A total of 192 different radiomic features were extracted from within cancer and healthy ROIs from the mp-MRI images. The dataset was divided into two parts, a training set (10 patients) and testing set (2 patients). Principle component analysis (PCA) was used for dimension reduction. A nonlinear support vector machine (SVM) using a Radial Basis Function (RBF) was used to generate a model for classifying as either tumor or healthy tissue. Finally, the RBF-SVM plus sigmoid combination was used to produce cancer probabilities of the entire peripheral zone. The testing data set was used to validate the optimized classification model and the accuracy, sensitivity, specificity of the model was measured.

Results

The optimized RBF-SVM classifier yielded an area under ROC curve of 0.902 for discrimination of cancer and noncancer voxels. The classifier achieved a sensitivity of 92.9%, specificity of 87.7% and accuracy of 92.9% for the patient 1 and the sensitivity of 91.4%, specificity of 83.7% and accuracy of 88.8% for the patient 2.

Conclusions

A CADx system using T2, DWI and DTI is described, which is able to differentiate cancer from noncancer with a high accuracy. The addition of a cancer probability model provides additional functionality for tumor heterogeneity interpretation.

RF2 A Cluster Randomized Controlled Trial of a Consumer Behavior Intervention to Improve Healthy Food Purchases From Online Canteens

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Background

Dietary risk factors are a leading cause of disease burden in Australia. Dietary habits established in childhood track into adulthood and predict future chronic disease including cancer. School canteens represent an ideal setting to implement public health nutrition strategies given their wide reach, and frequent use by children. Online canteens, where students order and pay for their lunch online, are increasingly prevalent, with the leading provider of online canteens servicing over 1200 schools nationally and processing over 13 million lunch orders per year. Such systems also provide an opportunity to implement, at scale and with fidelity, strategies to support healthy food purchases.

Aims

To assess the efficacy of a consumer behaviour intervention implemented in an online school canteen in reducing the kilojoule, saturated fat, sugar and sodium content of primary student lunch orders.

Methods

Ten NSW primary schools (2714 students) currently using an online canteen were recruited to a cluster randomized controlled trial conducted over a 2-month period. Intervention schools received a consumer behavior intervention integrated into their online menu (targeting menu labeling, healthy food availability, item placement and prompting). Control schools received no change to their online menu. Lunch order purchase data automatically captured by the online canteen were assessed using separate linear mixed models under an intention to treat framework with multiple imputation.

Results

Analysis of all available data ($n = 2714$ students) showed significant reductions in the average energy (-567 kJ; $P < 0.001$), saturated fat (-2.37 g; $P < 0.001$) and sodium (-228 mg; $P < 0.001$) content of intervention students' lunch orders.

Conclusions

The study provides strong evidence supporting the efficacy of a consumer behaviour intervention utilising existing online canteen infrastructure to encourage healthier purchasing from primary school canteens. Such an intervention may represent an appealing policy option as part of a broader government strategy to improve child public health nutrition.

RF3 Combinatorial Targeting of the c-KIT Receptor Tyrosine Kinase in Acute Myeloid Leukemia

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Background

Acute myeloid leukemia (AML) is the most common and aggressive form of acute leukaemia, with a 5-year survival rate of just 24%. Over half of all AML patients harbor activating mutations in tyrosine kinases, such as the receptor tyrosine kinases FLT3 (~33% of AML) and c-KIT (~7% of AML), which are associated with poor outcome. Clinical trials of targeted kinase inhibitors in AML have shown promise, however treatment resistance and relapse is common. We have previously identified that the DNA repair protein DNA-PK is activated downstream of FLT3; and targeting DNA-PK sensitises FLT3-mutant AML cells to tyrosine kinase inhibitors. The relative similarity between FLT3- and c-KIT-activated signaling pathways led us to assess the role of DNA-PK in c-KIT-mutant AML.

Aims

To evaluate DNA-PK as a therapeutic target in c-KIT-mutant AML.

Methods

The hematopoietic progenitor cell line FDC.P1 transduced with wildtype or mutant c-KIT, and a panel of AML cell lines (c-KIT mutant: Kasumi-1, c-KIT wildtype: HL-60, THP-1) were utilized. Drug toxicity was assessed using proliferation assays, and signaling pathways were profiled using mass-spectrometry phosphoproteomics.

Results

Targeted quantitative phosphoproteomics identified phosphorylation of DNA-PK at threonine 2645 in c-KIT mutant Kasumi-1 cells, indicative of DNA-PK activation. Accordingly, proliferation assays revealed that Kasumi-1 cells were more sensitive to DNA-PK inhibitors (NU7441, DNAPKiX) than wildtype c-KIT AML lines HL-60 and THP-1. Similarly, FDC.P1 cells expressing mutant c-KIT displayed significantly higher sensitivity to DNA-PK inhibition compared to controls. Inhibition of c-KIT signaling (Ibrutinib, FTY720) was synergistic with DNA-PK inhibition, selectively in c-KIT mutant cells. Discovery phosphoproteomics revealed that DNA-PK inhibitor treatment induced modulation of transcription and RNA metabolism ontologies in Kasumi-1 cells, providing insight into the oncogenic pathways regulated by DNA-PK beyond its canonical role in DNA repair.

Conclusions

DNA-PK activation may be a common event in receptor tyrosine kinase mutant AML and is a promising novel therapeutic target.

RF4 Identifying Classes of eHealth Literacy Among Magnetic Resonance Imaging and Computed Tomography Outpatients

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Background

Patient-centred communication is a key element of Australian Government-endorsed Optimal Cancer Care Pathways. The internet can provide patient self-determined, preference-sensitive access to health information. The implementation of such eHealth is a cornerstone of Australia's health reform agenda. eHealth literacy (i.e. ability to seek, find, understand, appraise and apply online health information) is necessary for patient engagement with eHealth. However, no studies have identified patient subgroups with similar eHealth literacy, who may benefit from support using online health resources.

Aims

This study sought to identify classes of patients reporting similar patterns of responses to an instrument measuring eHealth literacy.

Methods

Magnetic resonance imaging (MRI) and computed tomography (CT) are common procedures along the cancer care pathway. MRI and CT outpatients were recruited consecutively in one major public hospital waiting room. Participants completed a self-report questionnaire assessing their eHealth literacy, using the eHealth Literacy Scale (eHEALS). Emerging research suggests a three-factor eHEALS structure: awareness, skills and evaluation. Latent class analysis was used to identify eHealth literacy clusters.

Results

Of 268 eligible and consenting participants, 256 (96%) completed the eHEALS. Four latent classes were identified, which were labeled as low (21%), moderate (26%), high (33%) and very high (20%) eHealth literacy. Across all classes, participants were least confident in their awareness of, or ability to evaluate, eHealth resources, followed by skills.

Conclusions

Multiple eHealth literacy classes were identified, suggesting that eHealth literacy varies in this setting. Two of four latent classes, comprising nearly half of participants, represented lower eHealth literacy. These findings indicate that support is needed to assist patients to engage with eHealth. This support should target patient awareness and evaluation of online health resources. However, few high-quality intervention studies have evaluated eHealth literacy improvement interventions, indicating an evidence–practice gap requiring further research.

RF5 The Glue of Cancer Cell Life: Characterization of the Acellular Component of High Grade Serous Ovarian Cancer Identifies Potential Novel Drug Targets

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Background

The extracellular matrix (ECM) is a three-dimensional protein structure that confers architecture to tissues and dynamically interacts with cells to modulate their behaviour. The ECM is known to be deregulated in cancer and has been identified as a key mediator of cancer initiation, metastasis, and drug resistance. The development of drug resistance is a major factor contributing to the poor survival statistics of women with advanced ovarian cancer. Consequently, there is a dire need to develop more effective and durable treatment strategies.

Aims

To appreciate pathological changes in the ECM of ovarian cancer and identify potential ECM biomarkers as novel drug targets, we characterized and compared the matrisomes of human serous ovarian cancer and normal fallopian tube (purported site of cancer origin).

Methods

Human high grade serous ovarian cancer ($n = 8$) and normal fallopian tube ($n = 7$) tissue samples were homogenized and enriched for ECM proteins. Western blotting was performed to confirm efficiency of the enrichment process. The enriched ECM fraction was digested and analyzed using liquid chromatography tandem mass spectrometry. Mass spectrometry data was then

compared to an ECM database to identify the ECM protein composition of each sample. Identified proteins were compared with patient clinical history.

Results

Western blot confirmed ECM enrichment of human ovarian cancer and fallopian tube tissues. Comparative analysis of human ovarian cancer and normal fallopian tube matrisomes revealed significant differences in protein expression of glycoproteins, collagens and ECM regulators (Mann–Whitney *U* test; $P < 0.05$). Notably, we identified a novel biomarker, fibulin-3, which showed significantly higher expression in ovarian cancer tissue compared to normal control tissue. We are now investigating the role of fibulin-3 in normal fallopian tube/ovarian biology using a fibulin-3 knockout mouse model. These results will inform future studies aimed at understanding how this protein may be exploited in ovarian cancer.

Conclusions

By defining the extracellular matrix landscape of high grade serous ovarian cancer and its purported site of origin, we have established a set of potential ECM biomarkers that may be harnessed as novel drug targets in the future. Given the ECM is known to play a crucial role in cancer development, metastasis and chemoresistance, our ECM profiling data serves as a powerful platform to base further studies that may lead to a new paradigm of treatment for this deadly disease.

Manuscript

RF6 Advances in Electronic Portal Imaging Device-Based Real Time Assessment of Internal Anatomy to Guide Breast Cancer Radiation Treatment Under Deep Inspiration Breath Hold

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Background

Radiation coincidentally given to the heart while treating breast cancer with radiotherapy has been shown to increase the incidence of ischemic heart disease. To reduce this radiation, breast cancer patients are now often treated under deep inspiration breath hold (DIBH), which generally moves the heart further away from the target making it receive less radiation dose. DIBH is currently implemented by various means, all of which use a surrogate to assess the level of breath hold.

Aims

Our DIBH monitoring system directly assesses the internal anatomy of the patient. Using electronic portal imaging devices (EPID), which are part of all modern radiotherapy systems, the radiation beam treating the patient is captured to visualise and analyse the anatomy of the patient during treatment. We report on improvements to the system.

Methods

EPID images are collected during the treatment and analyzed in real time. A new analysis algorithm has been developed and tested. It uses a Hough transform algorithm for image rotation and compares lines of intensity values inside a definable region of interest in an incoming image to corresponding lines from the reference image. Normalized Cross Correlation is used to identify motion distance based on best match. Additionally, the user interface and data handling have been improved.

Results

Tested with and compared to 10 previously analyzed data sets the new algorithm found good agreement. The average absolute mean motion (± 1 SD) was 0.45 (± 0.13) mm with the average standard deviation (± 1 SD) of 0.72 (± 0.09) mm. The average of the maximum motion (± 1 SD) was 2.82 (± 1.70) mm.

Conclusions

The updated system proved reliable and fast enough for real time image analysis in continuous EPID based monitoring of DIBH treatments. The system works without any implanted or external markers, with no additional imaging equipment required and without additional radiation dose to the patient.

Manuscript

RF7 The Short-Term Effectiveness of Real-Time Video Counseling on Smoking Cessation Among Smokers Residing in Regional and Remote Areas

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Background

Real-time video counseling for smoking cessation can be delivered using software such as Skype and FaceTime directly to smokers at home. Such technology may be particularly beneficial to smokers living in regional and remote areas as it overcomes distance-related barriers to accessing smoking cessation treatment and provides visual interaction.

Aims

This study aims to assess the short-term effectiveness of real-time video counseling compared to telephone counseling or written materials (control) on smoking cessation and quit attempts in smokers living in regional and remote areas.

Methods

Participants were recruited via online and traditional methods into a three-arm, parallel group randomised trial. Eligible smokers needed to be aged 18 years or older, use tobacco daily, have access to video communication software (e.g., Skype), internet, telephone and email access, and live in regional or remote New South Wales. Participants completed an online baseline survey and were randomly allocated to either (1) real-time video counseling, (2) telephone counseling, or (3) written materials. Video and telephone conditions received identical content and call-back schedule of up to six counseling sessions. Participants completed a 4-months post-baseline survey.

Results

To date 562 participants have been recruited. Most are female (77.4%), married or living in a defacto relationship (55%), employed (60%) and live in inner regional areas (73%). The mean age is 43.4 years and mean number of cigarettes smoked per day is 19 (SD 9.4). In the video counseling condition, 75% made a quit attempt between baseline and 4 month follow-up, which compares favorably to the telephone counseling group (65%). The 7-day point prevalence abstinence finding at 4-months follow-up appears promising in the video counseling condition compared to the telephone counseling and written materials conditions.

Conclusions

These preliminary findings suggest that video counseling may be a promising approach for delivering smoking cessation support to those who live in regional and remote areas.

Author Manuscript

RF8 Microsampling as an Alternative Collection Method to Venous Blood to Quantify Capecitabine and its Metabolites by LC–MS/MS

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Background

Dose individualization of many anticancer therapies has been shown to significantly improve cancer outcomes by enabling optimum drug exposure or reducing major toxicity. Pharmacokinetic-guided dose individualization of capecitabine and 5-fluorouracil (5-FU) may be associated with an increase in overall survival and/or lower toxicity. However, this is difficult to achieve for remote patients where specialized facilities are unavailable. Volumetric absorptive microsampling collection as an alternative to venepuncture may facilitate this process in remote locations or in the home.

Aims

To evaluate the use of the Mitra microsampling device for its applicability in determination of capecitabine and its metabolites by LC–MS/MS.

Methods

Exact volume of whole blood (10 µL), obtained from volunteers, spiked with various analyte concentrations, was absorbed on Mitra microsampling devices and dried at ambient temperature for at least 3 h. Sample tips containing the absorptive pad were placed into the microcentrifuge tubes and acetonitrile containing stable isotope-labeled internal standards was added. Samples were sonicated, evaporated under vacuum and then re-suspended in 0.1% formic acid before injected into a Shimadzu 8060 LC–MS/MS. Chromatographic separation was on a Luna Omega Polar C18 (100 × 2.1 mm, 1.6 µm) column using gradient elution of 0.1% formic acid and acetonitrile.

Results

The intra- and interday imprecisions ranged from 3.0 to 8.1% and 6.3 to 13.3%, respectively, for capecitabine, 5'-deoxy-5-fluorocytidine, 5'-deoxy-5-fluorouridine and 5-FU. Accuracy ranged from 95 to 116%. LLOQ with imprecision of <18.8% and accuracy between 89 and 114% was 50 µg/L for 5-FU and 10 µg/L for all other analytes. Assays were linear from 50 to 50 000 µg/L for 5FU and 10 to 10 000 µg/L for all other analytes.

Conclusions

Microsampling with LC–MS/MS provides a method as reliable as conventional blood collection for capecitabine and metabolites. This may lead to less invasive and better timed sample collection for therapeutic drug monitoring supporting optimized cancer practice.

RF9 Assessing the Effectiveness, Feasibility and Acceptability of an m-Health Intervention to Improve the Nutritional Quality of Primary School Aged Children's Lunchboxes.

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Background

The prevention of certain cancers is strongly linked with maintaining a healthy body weight and consumption of a healthy diet such as adequate fiber, fruit and vegetables. School lunchboxes account for a third of a child's daily energy intake, however research indicates that energy dense foods are overrepresented in children's lunchboxes. Effective and scalable interventions are urgently required to improve the nutrition quality of children's lunchboxes and reduce the risk of chronic diseases, including cancer, later in life.

Aims

To assess the effectiveness, feasibility and acceptability of an m-health intervention, 'SWAP IT', targeting parents to swap what's packed in the lunchbox from discretionary 'sometimes' foods, to 'everyday' core foods.

Methods

A randomized controlled trial was conducted with 12 primary schools ($n = 1768$ students, mean age = 8.0 years) in New South Wales, Australia. Six schools received a multi-component intervention comprised of four strategies including; school nutrition guidelines, curriculum lessons, information pushed to parents (via the school app) and resources. Outcome measures taken at baseline and post-intervention (6-months) included mean energy (kJ) packed in lunchboxes assessed via observation, intervention feasibility and acceptability. Linear mixed models estimated the intervention efficacy.

Results

A nonsignificant reduction favoring the intervention group in the mean energy of foods packed within lunchboxes was observed between groups (-118.39 kJ, CI = -307.08 , 70.30 , $P = 0.22$). Viewing rate of the pushed messages to parents ranged from 39 to 100% of families over 10 messages. A large proportion (71%) of parents reported awareness of 'SWAP IT' and frequency of pushed messages was considered acceptable by 95% of parents.

Conclusions

'SWAP IT' shows promise in reducing the energy content of school lunchboxes and appears feasible and acceptable. A fully powered trial is warranted to determine the efficacy of the intervention on energy packed within the lunchbox in an effort to support cancer prevention.

RF10 Investigating ACVR1 and PI3K as Novel Therapeutic Targets in H3.1 K27M+ Diffuse Intrinsic Pontine Glioma

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Background

Diffuse intrinsic pontine glioma (DIPG) is a devastating childhood cancer for which there is no cure. Recent genetic analyses have revealed that 80% of patients harbor a genetic lesion to Histone H3 that sees the substitution of lysine 27 for a methionine (K27M) (H3F3A/H3.3 and HIST1H3C/H3.1). The loss of K27 leads to the loss of mono, bi or tri-methylation, likely to interfere with chromatin function, and activate transcription of numerous oncogenes. Unfortunately, there are no therapeutics active against the transcriptional programs unravelled by H3 mutations. However, activating mutations to ACVR1 occur in a quarter of all DIPG patients, with PI3K mutations co-occurring in more than half of these patients (56%), which represent promising therapeutic treatment targets in the absence of H3 targeted therapies.

Aims

This project aims to identify novel treatments for DIPG, and further elucidate the oncogenic pathways controlled by mutant ACVR1 and PI3K as potential therapeutic targets in DIPG.

Methods

Protein phosphatase 2A (PP2A) dephosphorylates transcription factors downstream of ACVR1. PP2A activity can be amplified using FTY720 and structural analogues, and thus may represent a novel therapeutic for DIPG.

Utilising DIPG patient derived cell lines harbouring H3 K27M mutations ($n = 6$), and $\pm$ ACVR1 and PI3K mutations, we have tested our chemical library of PP2A activating compounds alone and in combination with a novel PI3K inhibitor (PI3Kx), which is known to cross the blood–brain barrier.

Results

ACVR1 mutant DIPG cell lines were more sensitive to PP2A activation than ACVR1 wildtype DIPG cells. PI3K inhibition significantly reduced the growth of all PI3K mutant DIPG cell lines. Signal transduction analyses will confirm whether PP2A is a novel drug target downstream of ACVR1.

Conclusions

While there are currently no therapeutics active against the transcription programs unravelled by H3 mutations, these studies reveal a new targetable pathway that will be tested in preclinical models of DIPG.

PP1 The Role of Ion Channels in Melanoma

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Background

Melanocytes are cells derived from the neural crest region that during development, migrate to different parts of the body, including skin. Due to their lineage, melanocytes are believed to retain certain neuronal features throughout the development. Ion channels are pore-forming, integral membrane proteins that allow the passive transport of various ions across the cell membrane along their electrochemical gradient. In addition to their role in excitatory cells (i.e. neurons), ion channels are believed to have a crucial impact on all hallmarks of cancer.

Aims

We used a systematic approach to identify novel ion channels with a potential role in melanoma development and progression.

Methods

Bioinformatics approach was taken to identify the differentially expressed ion channel genes using publicly available datasets from Gene Expression Omnibus (GEO). Commonly upregulated ion channels among datasets were identified and considered for functional enrichment analysis using DAVID (Database for Annotation, Visualisation and Integrated Discovery). Short interference RNAs as well as pharmacological agents were recruited for further functional studies.

Results

Collective analysis of our results led to the identification of an ionotropic glutamate receptor NMDA (*N*-methyl-D-aspartate) type subunit as a novel candidate for further investigation in this study. Preliminary results revealed a potential role of this NMDA receptor in promoting melanoma cell survival and proliferation. Moreover, further pharmacological intervention showed enhanced sensitivity of cancer cells relative to normal melanocytes in vitro.

Conclusions

Our investigation unravels a potential role for a glutamate receptor subunit in pathogenesis of melanoma and may offer a promising therapeutic window complementary to current available methods.

PP2 A p53-Responsive MicroRNA Network Promotes Cancer Cell Quiescence

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Background

Cancer cells entering quiescence (G0 phase) are fundamentally resistant to death responses triggered by therapeutic agents and small pools of quiescent cells following treatment play decisive roles in cancer recurrence. Nevertheless, the mechanisms responsible for the regulation of cellular quiescence in cancer cells remain to be fully understood.

Aims

To characterize molecular signatures of quiescent cancer cells compared with cycling cancer cells and define the functional significance of the differentially expressed miRNAs in regulation of quiescence of cancer cells.

Methods

We employed a model system encompassing an mVenus-tagged p27 deletion mutant lacking the CDK-binding domain (mVenus-p27K-) together with a mCherry-tagged truncated mutant of human chromatin licensing and DNA replication factor 1 (hCDT1) (mCherry-hCDT1(30/120)) to label and isolate quiescent cancer cells. The purified quiescent cells were subject to small RNA sequencing. The miRNAs specifically upregulated in quiescent cells were further studied.

Results

We demonstrate that two p53-responsive miRNAs utilize distinct but complementary pathways to promote cancer cell quiescence through facilitating stabilization of p27. On the one hand, miRNA-27b-3p targets cyclin-dependent kinase regulatory subunit 1 (Cks1) leading to reduction in p27 polyubiquitination and subsequent degradation mediated by S-phase kinase-associated protein 2 (Skp2). On the other hand, miRNA-455-3p targets CDK2 associated cullin domain 1 (CAC1) that enhances CDK2-mediated phosphorylation of p27 at Thr187 necessary for Skp2-mediated p27 polyubiquitination.

Conclusions

Our results identify miRNA-27b-3p and miRNA-455-3p as important regulator of cancer cell quiescence in response to p53 and suggest that manipulating miRNA-27b-3p and miRNA-455-3p may constitute novel therapeutic avenues for improving outcomes of cancer treatment.

PP3 Australian AIEOP-BFM 2009 Acute Lymphoblastic Leukemia High-Risk Findings – Enrichment of IKZF1 Deletions and Other Curious Findings

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Background

Whole genome high density (HD) SNP microarray analysis provides valuable insights into the cancer genome, unveiling important prognostic findings and therapeutic targets.

Aims

We aim to identify prognostic related changes using HD SNP microarray in high-risk acute lymphoblastic leukemia (ALL). We also aim to validate rare findings with treatment change implications.

Methods

DNA was extracted from 52 high risk ALL patients with whole bone marrow (BM) taken at diagnosis and/or relapse. SNP-microarray was performed using the Illumina 850k CytoSNP and/or the Affymetrix HD SNP microarray platforms. Analysis was performed using the BlueGnome [Illumina] or ChAS [Affymetrix] software.

Results

Analysis of the Australian AIEOP-BFM 2009 ALL high-risk cohort using HD-SNP microarray identified important deletions, microdeletions and regions of loss of heterozygosity (LOH) not observed by standard clinical investigations. Analysis revealed a high proportion of IKZF1 deletions (25%) of which 54% were not detectable by other methodology. A curious case of near complete cnLOH and an ABL1-NUP214 fusion were also identified.

Conclusions

ALL is a complex disease revealing heterogeneous changes across the genome. Many changes, which are utilized for risk stratification and treatment guidance, are not currently identified at diagnosis. HD-SNP microarray is an effective tool, which increases the identification of such changes at diagnosis and relapse.

PP4 Why Targeted Therapies Don't Work?: Proteomic Characterization of Intratumor Heterogeneity of Human Endometrial Cancer

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Background

Endometrial cancer (EC) is one of the most frequently diagnosed gynaecological cancers worldwide, and its prevalence has increased by more than 50% over the last two decades. Despite the high incidence, intratumor heterogeneity is arguably the major force behind EC adaptation and progression that results in therapeutic failure. The existence of heterogeneity within the tumor, identified in different areas belonging the same patient, may influence the course of the disease and affect patient survival.

Aims

To characterize endometrial heterogeneity within the same patient at the proteomic level.

Methods

EC samples were obtained from women undergoing hysterectomies at the John Hunter Hospital, Newcastle using approved protocols. In this study, we isolated protein from tumor tissue samples obtained from different sites of EC from an individual patient (~4–5 samples/patient). We then performed comparative analysis using proteomic mass spectrometry approach to profile the protein composition of different areas within an EC tissue.

Results

Our proteomic analysis detected a combined total of 2469, 2696, 2393 and 2161 unique proteins in multiple EC tissues with a confidence corresponding to a false discovery rate <1% in four patients, respectively. We have identified insulin-like growth factor 2 receptor (IGF2R) as a protein biomarker that is commonly detected across all the samples collected from four EC patients. Furthermore, we revealed the list of unique proteins that exist only in a single EC location but are not present in other locations within the same tumor.

Conclusions

Our study has defined the proteome of EC and identified unique expression of proteins found in the different locations of EC within the same patient. Importantly, this study sets the foundation for further investigations into the mechanisms of endometrial heterogeneity. We propose that some of the proteins identified in our study represent potential novel EC drug targets that may change the paradigm of EC treatment.

PP2A Loss of Protein Phosphatase 2A Regulatory Subunit B55α Enhances Tumor Progression and Tamoxifen Resistance in Luminal Breast Cancer

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Background

Breast cancer is the most frequent female cancer. Recent genomic analyses of breast tumors have identified recurrent deletion of the PPP2R2A gene, which encodes the PP2A-B55α regulatory subunit of the serine/threonine protein phosphatase, PP2A. PPP2R2A deletion was most common in luminal B (ER+) tumors, a subtype of breast tumor, which responds poorly to current therapies. Our recent data has further shown that PP2A-B55α protein expression is low in aggressive breast tumors.

Aims

To determine the impact of PP2A-B55α loss on breast tumor aggression and response to therapies.

Methods

B55α protein expression was reduced using shRNA in tamoxifen sensitive MCF7 and resistant BT474 breast cancer cells lines. The effects of knockdown on cell proliferation, anchorage independent growth, migration, invasion, activation of signaling pathways, and sensitivity to tamoxifen and/or PP2A activating drugs, were assessed. In vivo tumor growth was determined by injecting BT474 cells in mammary fat-pad of mice.

Results

Knockdown of PP2A-B55α in BT474, enhanced transwell migration and invasion, accompanied by increased expression of epithelial to mesenchymal transition (EMT) marker proteins. Inhibition of PP2A-B55α also increased in vivo tumour growth of BT474 xenografts. In addition, PP2A-B55α knockdown induced increased proliferation, anchorage independent growth and resistance to

tamoxifen in MCF7 cells. Mechanistically, this was associated with increased pER α (S167). Importantly however, PP2A-B55 α -knockdown cells were sensitive to PP2A activating drugs. Furthermore, the addition of PP2A activating drugs sensitized the resistant cells to tamoxifen, suggesting a possible therapeutic option for patients with PP2A-B55 α -low tumors and/or drug-resistant tumors.

Conclusions

This study suggests that the inhibition of tumour suppressive PP2A-B55 α induces aggressive and therapy resistant breast cancer that may be targetable by PP2A activators.

PP6 Identification of New Causative Genes in Hereditary Colorectal Polyposis Syndromes

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Background

Hereditary colorectal polyposis syndromes represents around 2% of all diagnosed colorectal cancers (CRC). They are characterized by an abundance of colorectal polyps evolving to carcinomas if not treated. The most represented syndrome being Familial Adenomatous Polyposis (FAP) caused by a mutation in the APC gene. However, there is still a population with high risk of polyposis and CRC, with a heavy family history of CRC where no known causative mutation has been identified.

Aims

The aim of this study is to uncover potential causative genes leading to a polyposis phenotype. The identification of potentially pathogenic variants will allow to know which patients are at increased risk of cancer.

Methods

A cohort of 48 patients samples were selected based on three criteria: a strong family history of polyposis and/or CRC, a diagnosed polyposis phenotype and no mutation in the most common causative genes (APC and MUTYH) after genetic screening. DNA was extracted and we performed Whole Exome Sequencing to identify variants in the coding regions. Identified variants were filtered and classified based on predicted pathogenicity in silico.

Results

Two variants were significantly enriched in our cohort and predicted to be pathogenic: Frameshift variant c.220_221dupGG in SLAIN1 present in 19 patients and Stopgain c.910G>T in PRIM2 was present in 24 patients. Analysis also exposed the importance of DNA repair pathways in our cohort. Lastly,

copy number analysis showed deep deletion in six samples in DMTB1, a tumor suppressor gene.

Conclusions

Confirming the pathogenicity of those potentially deleterious variants would allow for a better and more comprehensive genetic screening in a clinical setting. This would help identifying patients with a higher risk of polyposis to treat and monitor them earlier and increasing their survival chance.

PP7 Characterization of a Novel Protein Target for Sphingosine-Based PP2A Activating Drugs in RTK+ AML

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Background

Acute myeloid leukemia (AML) is a very poor prognosis hematological neoplasm, with 5-year overall survival of <24%. The serine/threonine phosphatase tumor suppressor, PP2A, shows reduced activity in up to 78% of AML patients. Sphingosine based drugs; FTY720 and AAL(S) have been shown to reactivate PP2A. However, their mechanism of action is poorly defined, particularly in receptor tyrosine kinase mutant (RTK) AML. Here, we reveal the SBDS protein as a target of the PP2A activating drugs AAL(S) and FTY720.

Aims

The aim of our preclinical investigation was to determine the role SBDS plays in PP2A inhibition in RTK+ AML, and to characterize a novel role for SBDS in oncogenic signaling.

Methods

Cell culture models of RTK+ AMLs were employed alongside shRNA mediated molecular inhibition of SBDS expression and pharmacological PP2A reactivation with AAL(S). We have comprehensively assessed the activity of PP2A following molecular inhibition of SBDS, and rescued inhibition using recombinant SBDS. Phosphoproteomic profiling was employed to assess kinase signaling following PP2A reactivation. SBDS expression was assessed using primary AML samples and correlated with overall survival outcomes.

Results

Our studies show SBDS binds PP2A to inhibit its activity in both mutant FLT3 and c-KIT AMLs. Reactivation of PP2A following AAL(S) treatment inhibited SBDS activity. We reveal that reactivation of PP2A suppresses oncogenic signaling pathways, highlighting the potential for PP2A reactivation as a viable modality for therapeutic intervention. Characterization of SBDS expression in AML revealed for the first time, that it is commonly overexpressed (64.3%), and associated with reduced overall survival.

Conclusions

These data show that SBDS is a novel inhibitor of PP2A. Characterization of the cellular target of AAL(S) in vitro provides a mechanism of action for AAL(S) and FTY720. Targeting SBDS activity suppresses oncogenic signaling and induced AML cell death. Expression of SBDS in myeloblasts may prove a marker for reduced survival in patients.

PP8 Development of Novel Model Systems for the Study of Resistance to Targeted Therapies in Acute Myeloid Leukemia

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Background

Acute myeloid leukemia (AML) is a lethal blood disease resulting from the malignant transformation of a hematopoietic stem/progenitor cell. Currently, two-thirds of AML patients achieve complete remission (CR) with high dose chemotherapies. However, up to 80% of patients relapse and die from an even more aggressive drug resistant leukemia. AML relapse results from the clonal evolution of malignant founding clones, which are induced, in part, by chemotherapy itself. Internal tandem duplications of FMS-like tyrosine kinase-3 (FLT3/ITD) mutations are found in approximately 23% of de novo AML patients and are associated with poor prognosis due to higher rates of relapse, shorter relapse-free survival, and decreased likelihood to respond to therapies at relapse.

Aims

Two tyrosine kinase inhibitors (TKI) have recently entered the clinic (Sorafenib and Midostaurin) for FLT3 mutant AML. Nevertheless, many patients form resistance to these therapies due to the emergence of additional activating point mutations to the kinase domain of FLT3. Therefore, the aim of this study is to unravel compensatory signalling pathways that lie downstream of this 'double-mutant receptor', to help uncover the mechanism underpinning AML resistance and new drug targets.

Methods

Using factor-dependant mouse myeloid progenitor cells, we have generated factor-independent AML-like myeloblasts via retroviral transformation to express the most common human AML FLT3 mutations (FLT3-WT, -ITD, -D835V, -D835Y, -ITD/D835V&Y), and conducted growth and survival assays.

Results

Our analyses revealed the double mutants (ITD/D835) to be resistant to cytarabine, daunorubicin, sorafenib and midostaurin, the therapies most commonly used in the clinic. Currently, we are performing high-resolution comparative and quantitative proteomic and phosphoproteomic analyses on these FLT3 mutant cells to identify new drug targets for resistant cells.

Conclusions

Future work will test these therapies in resistant patient samples ex vivo and in vivo. This work will help us uncover novel drug targets to reduce treatment relapse and provide options for relapsed patients.

PP9 Heterogeneous Nuclear Nucleoprotein A3 (hnRNPA3) is a Novel Binding Partner for Brain and Acute Leukemia, Cytoplasmic (BAALC)

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Background

Acute myeloid leukemia (AML) is the most common acute leukemia in adults and has a 5-year survival rate of 2–9% in adults aged over 65 years. Despite advances in treatment of AML over 50% of older adults die within two months of treatment and most will relapse. Primary resistance to chemotherapeutics increases with age and affects 33% of patients under the age of 65 and more than 57% of patients over 75 years. Overexpression of the brain and leukemia, cytoplasmic (BAALC) gene is associated with increased incidence of primary

refractory AML. We have previously shown that BAALC overexpression increases AML cell proliferation and decreases cell sensitivity to chemotherapeutics. However, precisely how overexpression of BAALC is controlling sensitivity to chemotherapeutics is unknown.

Aims

The main aim of this study was to identify how BAALC overexpression controls AML cell proliferation and survival.

Methods

BAALC was overexpressed in a panel of AML cells, and reciprocal co-immunoprecipitations were performed to identify proteins that interact with BAALC. Immunofluorescence was performed to confirm the interaction with hnRNPA3. To elucidate the cellular functions mediated by this interaction, the expression of hnRNPA3 was reduced via siRNA transfection. Following this, effects on proliferation and survival were examined (cell counts, resazurin, Annexin assays).

Results

We have identified hnRNPA3 as a novel binding partner of BAALC, and have shown that this interaction is a possible mechanism in the BAALC-mediated control of AML survival and proliferation.

Conclusions

BAALC is a potential target for the treatment of AML. Since BAALC has restricted expression in normal cells, drugs that target BAALC or its binding partners may present more cancer cell specific effects than existing chemotherapeutics.

PP10 What is the Best Way to Measure Blood Cell Mean Telomere Length?

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Background

Telomeres are a robust biological marker of cellular aging, with short blood cell telomere length considered a genetic risk factor for hematological disorders including myelodysplastic syndrome, AML and CLL. Despite newer technologies, the measurement of mean telomere length by Southern blotting remains the gold standard.

Aims

In this study, we aim to improve reliability of the method by comparing DNA extraction processes, digestion and amounts.

Methods

Genomic DNA was extracted from whole blood using commercial kits (Qiagen) or from red cell lysed-white cell pellet by the salt-extraction method. DNA was digested overnight at 37°C using either a two (RsaI; MspI) or six (HhaI; HinfI; MspI; HaeIII; RsaI; AluI) restriction endonuclease combination (Promega). Amount of DNA (1.0, 1.5, 2.0 and 2.5 µg) in the digestion was also compared. Telomeres were detected with a DIG-labeled oligonucleotide probe, visualized by chemiluminescence and analyzed with ImageQuant TL v8.1.0.0 software (GE Healthcare Life Sciences). Mean telomere length was calculated using the formula $\Sigma(MW_i \times NI_i) / \Sigma NI_i$. Statistica was used for all statistical analyses.

Results

Between blot variation was 6.5% ($n = 7$). Samples digested with two endonucleases had a mean telomere length 12% longer than those digested with 6 endonucleases ($n = 17$; $P = 0.007$). When comparing amount of DNA, there was a significant difference in mean telomere length between samples extracted with the in-house salt-extraction method ($P = 0.03$) but not DNA extracted using commercial kits ($P = 0.33$).

Conclusions

Measurement of mean telomere length is more reliable and reproducible when using a commercial DNA extraction kit and digestion with the six restriction endonuclease combination. While the amount of DNA makes no difference under these conditions, a smaller volume is advisable for practical reasons. Telomere length analysis by Southern blotting is a robust and consistent biomarker assay suitable for implementation in both clinical and research settings.

PP11 Differences in Modified Tetraspanin Extracellular Vesicle Nucleic Acid Cargo Show Promising Signs for Prostate Cancer Biomarkers

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Background

Biomarkers for prostate cancer are required to improve decisions regarding treatment options for newly diagnosed men. The tetraspanins CD151 and CD9 show promise as biomarkers, however still lack the accuracy required. Tetraspanins are commonly found in the membrane of extracellular vesicles (EVs), however it is currently unknown if changes to CD151 and CD9 expression seen in prostate cancer lead to altered incorporation of RNA into EVs from tumor cells potentially providing the biomarkers required for prostate cancer.

Aims

To identify EV cargo specifically incorporated as a response to altered tetraspanin expression that can be used to enhance the potential of tetraspanins as prognostic biomarkers for prostate cancer.

Methods

CD151 was increased and CD9 expression was decreased in RWPE1 (normal prostate cells) increased in PC3 (bone metastasis from prostate cancer) to manipulate resultant incorporation of CD151 and CD9 into EVs from these cells. EVs were collected by ultraconcentration from cell culture media devoid of supplements after 48 h. Total RNA was extracted and evaluated using bioanalyzer chips. Affymetrix Human Transcriptome arrays were used. Transcriptome analysis console was used for visualisation.

Results

Increasing the expression of the metastasis suppressor CD9 in prostate cancer cells resulted in increased incorporation of CD9 in EVs and the differential incorporation of 2532 transcripts. Whereas decreasing CD9 and increasing CD151 expression in RWPE1 cells and the resultant EVs altered the incorporation of 486 and 50 transcripts respectfully, into the EVs. The top decreased transcript in CD151 high EVs correlates to previous data found in metastasis EVs.

Conclusions

Tetraspanins CD151 and CD9 are involved in the selection of RNA cargo into EVs from prostate cancer cells. Several potential biomarkers were identified. Further work is ongoing investigating the function of these transcripts in EVs and confirming their biomarker potential in patient serum samples.

PP12 Methylation Specific Droplet Digital PCR Accurately Quantifies BCAT1 Allele in Colorectal Cancer Patients

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Background

Epigenetic methylation has emerged as a potential next generation molecular screening biomarker for colorectal cancer (CRC). Methylation of 'CpG Islands' in promoter regions plays an important role in the regulation of genetic transcription and are an early change in the pathway to malignancy. Methylation of the BCAT1 gene has been previously identified as a potential biomarker in CRC. Droplet digital PCR (ddPCR) technology has many valuable attributes that have rarely been utilised in the detection of epigenetic DNA methylation. We aim to utilize the high sensitivity, absolute quantification and the ability of multiplex assays that ddPCR provides to assess potential methylated biomarkers in CRC.

Aims

To investigate the utility of droplet digital methylation specific PCR in the detection of epigenetic biomarkers in colorectal cancer.

Methods

Samples were collected from the resected specimens of 14 patients with CRC. DNA was extracted from normal proximal-colonic mucosa and tumor tissue then bisulfite treated prior to methylation-specific PCR (MSP). MSP analysis was performed utilising the Bio-Rad ddPCR platform. A duplex BCAT1 gene assay was performed utilizing custom designed methylation specific primer and probe sequences. The methylation ratio (MR) was calculated and an ROC curve was utilized to determine the optimal cutoff point of the test.

Results

Droplet digital PCR detected a significant difference in the number of methylated alleles between tumor and proximal colonic tissue. ROC curve of the BCAT1 gene had an AUC of 0.844 ($P = 0.001$, standard error 0.075, 95% CI 0.697–0.992). At the optimal threshold calculated by the ROC curve (MR 0.174) the sensitivity was 73.3% and the specificity was 94.3%.

Conclusions

Droplet digital PCR is potentially very useful for epigenetic methylation analysis since it is sensitive at detection on low-input samples. Our results indicate that the methylated BCAT1 gene could be used in a panel of biomarkers for early detection of CRC.

PP13 Molecular Characterization of Treatment Resistance in FLT3 Mutant Pediatric Acute Myeloid Leukemia

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Background

Acute myeloid leukemia (AML) is the second most common form of leukaemia in children but responsible for the most number of leukemia-associated deaths. The majority of children achieve an initial chemotherapeutic response however, 20% will relapse and die. Relapse is common in patients harboring FLT3-internal tandem duplication mutations (FLT3-ITD) and diagnosed in 20% of pediatric AML cases. FLT3-ITD+ patients and those that do not respond to anthracycline- and cytarabine-based chemotherapy are recommended for hematopoietic stem cell transplantation (HSCT). The role HSCT plays in the treatment and survival of pediatric AML remains uncertain. Personalized treatment strategies targeting FLT3 appears to be the best way forward however, resistance to promising new targeted therapies is a major issue.

Aims

To define the mechanisms underpinning the development of resistance to cytarabine and anthracycline based chemotherapies in FLT3-ITD mutant AML cells. We hypothesize that analysis of posttranslational modifications coupled to a proteogenomic approach will identify new drug targets to reduce relapse.

Methods

Cytarabine and daunorubicin resistance was induced alone and in combination using a pediatric FLT3 mutant AML cell line. A series of western blots were conducted on resistant cell lines, followed by DNA halo assays. Currently,

comparative and quantitative proteomic profiling coupled to analysis of acquired somatic mutations using Next Generation Sequencing is underway. Functional analysis of gene and pathway alterations will be validated using molecular inhibition and drug repurposing studies and validated in a cohort of primary AML samples at diagnosis and following resistance.

Results

ABCG2 protein expression was significantly increased in resistant cells. Cytarabine resistant cells showed decreased reliance on phosphotyrosine signaling, while both showed increased acetylation of histone H3. In congruence with increased acetylation of H3, DNA halo preparations showed decreased chromatin compaction and a remarkable level of sustained oxidative DNA damage contributing to the clonal evolution of these cells during treatment.

Conclusions

This project aims to provide the first proteogenomic characterization of chemotherapy resistance in pediatric FLT3 mutant AML. This high-resolution approach will help to correlate acquisition of gene mutations and protein expression with resistance.

PP14 Mouse Models to Investigate the Genetic Mechanism Underlying the Development of Non-Small Cell Lung Cancer

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Background

Non-small cell lung cancer (NSCLC) accounts for 80–85% of all lung cancers and contributes significantly to cancer-related morbidity and mortality in Australia and worldwide. Cigarette smoking accounts for ~85% of all LC cases. It alters the expression of genes responsible for normal cellular function. The current diagnostic techniques fail to detect NSCLC at an early stage due to the poor understanding of genetic events leading to the development of NSCLC. Conducting genome-wide studies can identify novel mutations responsible for the carcinogenesis that could be used as diagnostic markers.

Aims

Developing a mouse model for NSCLC. Identifying genetic alterations linked to the development of LC using long-term tobacco carcinogen/cigarette-smoked wild-type mouse models and validate them in short-term mouse models and human samples.

Methods

A/J mice were administered a tobacco carcinogen, NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone) and exposed to cigarette smoke (CS) for varying periods. Whole genome sequencing (WGS) is being performed on tumor and tails from the long-term model. The results will be validated in short-term mouse models and human samples using targeted sequencing.

Results

Our group has established a novel short-term mouse model where 100% of mice exposed to NNK and 8-weeks of CS followed by 8-weeks of air rest develop adenomas resembling human bronchoalveolar adenomatous hyperplasia. These

tumors eventually progress to large adenocarcinomas when the NNK treated mice are exposed to 36-weeks of CS followed by 27-weeks of air rest.

Conclusions

Our novel mouse model recapitulates the crucial pathophysiological features of human LC. We anticipate that data from WGS would identify genetic alterations in pre-neoplastic stages of LC that can be translated into early diagnostic tests.

PP15 Oncogenic upregulation of the long noncoding RNA5

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Background

Through bioinformatics analysis of publically available RNA sequencing datasets, we found that lncRNA5 was commonly unregulated in diverse types of human cancers compared with corresponding normal tissues

Aims

To examine the functional significance of the lncRNA5 upregulation in the pathogenesis of cancer.

Methods

Human cancer cell lines carrying an inducible lncRNA5 shRNA system in response to doxycycline (DOX) were used as tools to investigate the effect of lncRNA5 silencing on cell proliferation and survival. The results were confirmed by overexpression of lncRNA5. Comparative RNA sequencing analysis was carried out to identify potential downstream targets of the lncRNA5. RNA pulldown, followed by mass spectrometry, and RNA immunoprecipitation were used to interrogate RNAs and proteins binding to the lncRNA5. The roles of identified lncRNA5 binding partners in the lncRNA-mediated cell survival and proliferation were tested by combined knockdown and overexpression.

Results

Our results confirmed that the lncRNA5 was frequently upregulated in cancer cells. Functional investigation revealed that the lncRNA5 promoted cancer cell survival and proliferation. This was closely associated with p53 protein stabilization and its interaction with G6PD, a p53 binding partner.

Conclusions

The lncRNA5 plays an important role in cancer development and progression. This is, at least in part, due to its inhibitory effect on p53 and stimulatory effect on G6PD activity.

PP16 Intracellular Oxidative Stress Modulates FLT3 Regulatory Proteins Contributing to Oncogenic Signaling in Acute Myeloid Leukemia

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Background

Treatment of acute myeloid leukemia has remained unchanged over the last 35 years, with 60% of patients relapsing after initial treatment success using chemotherapies. Acute myeloid leukemia (AML) cells produce high levels of ROS, which in-part contributes to resistance and relapse. FLT3-ITD (Internal Tandem Duplication) mutations are the most common mutation in AML, and are associated with the overproduction of ROS causing increased genomic instability through the oxidation of DNA bases. Importantly, ROS oxidizes and inactivates key proteins that regulate growth and survival.

Aims

To determine the cooperative mechanisms of oxidation and cellular metabolism underpinning leukemogenic growth and survival signaling.

Methods

Bone marrow biopsies from AML patients harboring FLT3-ITD+ or wild-type FLT3 were subjected to high-resolution quantitative proteomic, phosphoproteomic and Redox sequencing. From this novel, clinically relevant therapies targeting oxidative stress and FLT3-ITD were assessed in cell line models of AML. ROS flow-cytometry assays, western blotting, cytotoxicity

assays and targeted OxPRM mass spectrometry were used to investigate the Redox and proteomic changes occurring with treatment.

Results

Patients expressing FLT3-ITD mutations showed significantly increased expression of NADPH oxidase 2 (NOX2) and associated proteins, directly responsible for ROS production. Oxidation and inactivation of tumor suppressor proteins particularly, the protein tyrosine phosphatases (PTPs) downstream of mutant FLT3 were seen compared to AML patients expressing wild-type FLT3. Proteins important in maintaining cellular homeostasis, such as antioxidants were differentially dysregulated between patient subtypes supporting the notion of REDOX dysfunction in FLT3-ITD+ AML. Reducing intracellular oxidative stress levels by inhibiting NOX2 in combination with FLT3 inhibitors currently in clinical trials, reactivated intrinsic cellular defence systems, inducing selectively synergistic cell death. Importantly, analysis of AML cells grown under conditions mimicking the bone marrow microenvironment, enhanced the anti-leukemic efficacy of our therapies.

Conclusions

These studies suggest a mechanism of cooperation between oncogenic kinases, metabolism and oxidative stress to reveal a novel treatment paradigm currently under preclinical evaluation.

PP17 Quantitative Proteomics for the Early Detection of Colorectal Cancer

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Background

Colorectal carcinoma (CRC) is a common form of cancer worldwide. The natural history of progression of adenomatous polyps to CRC has been well described by the adenoma-carcinoma sequence. Screening is most commonly conducted by stool-based tests, however participation is suboptimal. This project aims to discover a plasma-based protein that could be used for the early detection of colonic adenoma and carcinoma using a combination of targeted and shotgun mass spectrometry.

Aims

The discovery of a plasma-based protein to detect adenoma and early colorectal carcinoma using a combination of shotgun proteomics for plasma analysis and data dependent acquisition for histological analysis.

Methods

The project will be conducted in three phases: the first using patient's plasma samples to identify a protein predictive of colonic adenomas and colorectal cancer with a label free mass spectrometry (MS) approach. The second will be conducted using histological sampling of healthy bowel tissue, early-stage precancerous polyps and adenocarcinoma using an iTRAQ-labeled MS approach. The third will be a validation set using parallel reaction monitoring to perform a comparative and quantitative analysis of proteins identified from phases one and two.

Results

The project is in its early stages, however expected results would be discovery of a low abundant protein differentially expressed in early cancer and adenoma using a combination of label free and label based proteomics. The project is unique in the methods of sample collection, storage of plasma in optimised blood collection tubes as well as the combination of differing MS technologies.

Conclusions

The projects aims to discover a plasma protein or panel of proteins that could be used as a screening tool for colonic adenoma or carcinoma using a translatable method, which could significantly improve population compliance to screening regimes, therefore having a flow-on affect to lower colorectal cancer morbidity and mortality.

PP18 Does Head and Neck Lymphedema Impact Swallow Function?

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Background

Head and neck lymphedema (HNL) is a common side effect of head and neck cancer (HNC) treatment. It's evidenced by the abnormal swelling and accumulation of protein rich fluid within the interstitial spaces; and may occur internally (i.e. within the oral cavity, pharynx or larynx), externally (i.e. on the face or neck), or as a combination of both. HNL may contribute to long-term dysphagia; however, the extent of the impact on swallowing is largely unknown.

Aims

To examine the prevalence of HNL in HNC patients posttreatment, and the impact of HNL on swallow function.

Methods

Fifty-six HNC patients, 1–3 years posttreatment, were prospectively recruited. Internal HNL was assessed via nasendoscopy and rated with Patterson's Scale. External HNL was rated with the MD Anderson Lymphedema Rating Scale. Aspiration status was rated with the Penetration–Aspiration Scale and diet status was scored with the Functional Oral Intake Scale. Relationships between HNL and swallowing were examined using Spearman's rho.

Results

Patients were predominately male (84%), had oropharyngeal tumors (64%), were treated with chemoradiotherapy (63%), and were assessed a mean of 18.7 months posttreatment. The vast majority of patients had some form of HNL (98%). Combined internal and external HNL was observed in 39%; while 57% had internal HNL only, and 2% had external HNL only. Penetration/aspiration was observed in 30%, and 85% required some form of ongoing diet modification. A significant but fair correlation was found between penetration/aspiration and the severity of internal HNL ($r = 0.30$). Significant but fair relationships were also observed between diet status and the severity of internal ($r = 0.29$) and external ($r = 0.41$) HNL.

Conclusions

HNL is highly prevalent in HNC patients posttreatment. HNL that is more severe may contribute to long-term dysphagia; with patients more likely to experience penetration/aspiration and require ongoing diet modification.

PP19 Using the Behavior Change Wheel to Inform an App-Based Intervention to Increase Parents' Packing of Healthy Lunchbox Foods for Children Attending Center-Based Childcare

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Background

The current dietary intake of Australian children is largely inconsistent with National Dietary Guidelines. As childhood dietary habits can track into adulthood, improving children's diets has the potential to reduce the risk of chronic diseases, such as cancer. Children attending childcare services can consume up to two-thirds of their daily dietary intake via foods packed in the lunchbox. Current research indicates that children's lunchboxes contain inadequate quantities of vegetables and excessive discretionary foods. Given this, there is a need for the development of effective interventions to support parents in packing healthy lunchbox foods.

Aims

To report on the application of the Behavior Change Wheel in the development of a parent-based healthy lunchbox intervention delivered via childcare App technology.

Methods

The Behaviour Change Wheel (BCW) was chosen as the theoretical framework to support the design of the intervention. A literature review and semi-structured interviews with parents were undertaken to identify parental barriers to packing healthy lunchboxes. The findings were subject to behavioral analysis, using the BCW Framework. Relevant behavior change techniques were identified to inform the development of intervention strategies.

Results

Parental barriers identified included lack of knowledge, time, cost, child food preferences and lack of prompts and motivation. An intervention consisting of three key strategies incorporating the use of nine behavior change techniques was developed. Existing features of the App technology such as the ability to regularly provide information, online links and use of push notifications, were utilized as modes of delivery.

Conclusions

This research provides insights into parental barriers to packing healthy foods and describes how a behavior change framework can inform the development of a childcare parent-based intervention. The resulting intervention is the first to employ the use of childcare App technology to directly reach and engage parents and improve food packed in child lunchboxes.

PP20 Haem-Fit: A Pilot Implementation Trial of a Hospital Exercise and Wellness Program for Hematology In-Patients

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Background

Hematology in-patients often undertake extended admissions, complex treatments and experience adverse events that can affect the uptake and adherence of exercise. Despite being a vulnerable group, studies conclude exercise is generally safe, feasible and efficacious in minimising skeletal muscle atrophy and improving quality of life.

Aims

The aim of this study was to evaluate the rate of patient adherence to a structured in-patient exercise program and the difference in lean muscle mass between admission and discharge.

Methods

This pilot study was conducted in a 12 bed haematology unit over 12 weeks (February–May, 2018). Patients with a hematological malignancy, an expected length of stay ≥ 7 days and able to undergo impedance testing were eligible. On admission and discharge consented participants completed Hospital and Anxiety Depression Scale (HADS), and a Quality of Life Questionnaire (QLQ-C30). On admission, each participant consulted with a physiotherapist and dietician where exercise targets and nutritional goals were set and integrated into participants prescribed plan of care. On discharge participants completed an exercise diary and evaluation.

Results

Thirteen of 16 eligible patients consented (81% accrual rate). Two participants were lost to follow-up and only eight of 11 participants completed their exercise diaries. All participants undertook some of the exercises, but only three achieved $\geq 50\%$, giving an overall adherence rate of $54.37 \pm 26.6\%$. All but one participant lost significant muscle mass (average = $-4.62 \pm 3.12\%$, $P = 0.003$, $n = 10$), with four participants losing $>6\%$. Although there was no significant improvement in QLQ-C30 or HADS scores, feedback from patient satisfaction survey was overwhelmingly positive.

Conclusions

We were able to successfully implement an exercise program that could be performed by in-patients. Although patient adherence and satisfaction with the program was high, muscle loss was still significant. Exercise modifications may be required to improve patient outcomes.

PP21 Feasibility and Acceptability of ICAN QUIT in Pregnancy Multicomponent Implementation Intervention and Research Design for Australian Indigenous Pregnant Women: A Pilot Cluster Randomized Step-Wedge Trial

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Background

Many health providers (HPs) lack knowledge, confidence, optimism and skills in addressing smoking with pregnant women. Smoking in pregnancy is a key challenge for indigenous health.

Aims

Explore the feasibility and acceptability of a co-designed multicomponent intervention at Aboriginal Medical Services (AMSs) in culturally targeted pregnancy-specific smoking cessation care (SCC).

Methods

Randomized step-wedge cluster design. The Indigenous Counselling And Nicotine (ICAN) QUIT in Pregnancy Trial was implemented in six AMSs across three Australian states. HPs were provided an educational resource package, including live interactive webinars, treatment manual, patient resources, carbon monoxide (CO) meter and oral Nicotine Replacement Therapy (NRT). Feasibility was assessed through recruitment and retention rates of pregnant women and HPs. Qualitative interviews with staff post-trial explored acceptability of intervention and study design related to Capability, Opportunity and Motivation from the Behavior Change Wheel.

Results

Pregnant women ($n = 22$; 47% eligible) and HPs ($n = 50$; 54% eligible) were recruited with 12-week retention rates of 77 and 40%, respectively. Self-reported 12-week 7 day point-prevalence abstinence was 13.6% ($n = 3$), and validated with CO readings ≤ 6 ppm. Staff interviews highlighted the importance of provision of resources, which increased capability and opportunity, restructured the environment, and provided social comparison and modeling. Staff were motivated by greater engagement with pregnant women, and the women's reductions in CO readings. The implementation at the AMSs improved organisational capacity to engage with smoking cessation. Staff reported changes to their routine practice that were potentially sustainable. Recommendations for improvement to the implementation of the intervention and research included reducing training length and study requirements and amending the step-wedge design.

Conclusions

ICAN QUIT in Pregnancy was a pilot study with the ability to engage Indigenous women in SCC. The intervention was feasible to implement and acceptable to most staff of AMSs in three states, with modifications to design recommended.

PP22 2018 Update: The Recruitment and Consultation Process for a National Evidence-Based Trial on Smoking Cessation Care for Pregnant Aboriginal and Torres Strait Islander Women—The SISTAQUIT® Experience

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Background

Pregnancy is an important window of opportunity to help smokers quit. Evidence-based, culturally appropriate smoking cessation care (SCC) is required to lower smoking prevalence (45%) among pregnant Indigenous women. SISTAQUIT (Supporting Indigenous Smokers To Assist Quitting), a cluster randomized controlled trial at Aboriginal Medical Services (AMS) and GP practices, compares normal care versus culturally appropriate SCC training to health providers (HPs) to determine if training improves quit outcomes. More evidence is needed regarding the most effective strategies to recruit research sites.

Aims

To provide an update on the Australia-wide recruitment and consultation phases for SISTAQUIT.

Methods

Peak bodies and AMS were contacted in NSW, QLD, WA, SA and the NT, and successive waves of sites were recruited. Strategies included mail-out of trial information to CEOs, phone calls and emails to services, newsletters, presentations at seminars, social media ((Recruitment) phase I). Interested sites were queried for eligibility regarding trial inclusion criteria (phase II). Eligible

sites received organizational information and consent forms, the site Research Facilitator position description, and site reimbursement information after jurisdictional ethical approvals were received (phase III).

Results

The opportunity to access whole of service training in SCC for pregnant women an important attractor for sites. Face-to-face contact facilitated interest, assisted by regular email and phone contact. Site recruitment was highly dependent upon the timing and ability to achieve ethical approval for sites within each jurisdiction. Recruitment was facilitated by clarification of site responsibilities and the reimbursement available for successful recruitment and follow up of trial participants.

Conclusions

A wide variety of communication approaches were required to recruit AMS facilities to participate in the SISTAQUIT trial. Coordination of recruitment strategies with the ethical approval process is essential. Clarity regarding site roles and reimbursement enhanced site recruitment.

PP23 Nicotine Replacement Therapy (NRT) Uptake and Utilization as a Smoking Cessation Aid: National and International Comparisons to Assist Implementation of SISTAQUIT®—An Evidence-Based Trial on Smoking Cessation in Pregnant Aboriginal Women

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Background

Pregnancy is an important window of opportunity to help smokers quit. SISTAQUIT (Supporting Indigenous Smokers To Assist Quitting), a cluster randomized controlled trial at Aboriginal Medical Services (AMS) and GP practices, compares normal care versus evidence-based culturally appropriate smoking cessation care (SCC) training to health providers to determine if training improves quit outcomes. NRT utilization by pregnant trial participants collected during the trial requires contextualization with usage nationally and internationally.

Aims

Primary Aim: To describe Australian usage of NRT, particularly for Aboriginal and/or Torres Strait Islander people. Secondary aim: To describe NRT usage in select International countries, particularly for First Nation peoples and/or pregnant women.

Methods

Longitudinal Australian NRT usage by state and other Australian usage data were examined for trends against Pharmaceutical Benefits Scheme (PBS) legislation. A semi-structured review for publications regarding NRT usage by

Australian Aboriginal and/or Torres Strait Islander people was performed. Comparisons against international NRT usage in selected countries (e.g. UK, USA, and New Zealand) were undertaken, to examine international trends in NRT usage, particularly for First Nation peoples and/or pregnant women.

Results

Preliminary results of the NRT usage in Australia will be presented, along with comparisons of utilization in selected countries with an emphasis on NRT uptake by First Nation peoples and/or pregnant women.

Conclusions

More research is needed to characterise recent NRT usage patterns across Australia, particularly for pregnant Aboriginal and Torres Strait Islander pregnant women.

PP24 Online Versus Traditional Recruitment of Rural Smokers into a Cessation Trial

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Background

Traditional recruitment methods include print (e.g. newspapers), broadcast advertising (e.g. radio, television) plus word of mouth while online recruitment methods include social media (e.g. Facebook, Twitter), e-mail and other online promotions. However, no studies have compared online and traditional methods for recruiting a regional and remote population into smoking cessation support.

Aims

The aim of this study is to examine whether participant demographic characteristics are associated with method of recruitment (i.e. online, traditional) into a smoking cessation trial.

Methods

Participants are being recruited into a randomized trial that provides smokers different forms of behavioral support. To be eligible to participate smokers need to be aged 18 years or older, use tobacco daily, have access to video communication software (e.g. Skype), internet and telephone access, an email address and live in a regional or remote area of New South Wales. Various online (e.g. Facebook ads, Twitter) and traditional methods (e.g. newspapers, posters) are being used to recruit eligible participants.

Results

To date 562 participants have been recruited. The mean age of participants is 43.4 years and the majority are female (77%), married or living in a defacto relationship (55%) and live in an inner regional area (73%). Most participants have been recruited through online and social media (>87%) while more than a tenth heard about the study via traditional means (e.g. newspaper, flyers, word of mouth, radio). The characteristics associated with recruitment method will be presented.

Conclusions

The preliminary findings suggest that online social media is a viable approach for recruiting regional and remote participants into a smoking cessation trial.

PP25 Connectivity of Real-Time Video Counseling Versus Telephone Counseling for Smoking Cessation

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Background

Real-time video software (e.g. Skype, FaceTime) is widely available and facilitates virtual face-to-face communication. However, quitlines have not implemented real-time video counseling into routine practice. A potential barrier may be the perceived connectivity of video communication technology compared to telephone.

Aims

This study aims to investigate the connectivity of video sessions compared to telephone sessions delivered to smokers in regional and remote areas.

Methods

Participants are from a randomized trial in which two arms offer (a) real-time video counseling or (b) telephone counselling to assist smokers to quit. Depending on their allocation, participants are offered up to six video or telephone sessions. On completion of each video or telephone session, the smoking cessation advisor records the quality of that call (e.g. connectivity, audio quality, picture quality (video only)).

Results

To date 373 video counselling sessions and 476 telephone counselling sessions have been completed. Preliminary findings show there is adequate connectivity of the video intervention in regional and remote locations with there being no echoing noise in more than 96% of video sessions. In approximately 86% of video sessions, there was no difficulty seeing and hearing participants and no loss of internet connection. In the telephone counseling condition, more than

95% of telephone calls had no echoing noise, no difficulty hearing the participant and no loss of telephone line connection.

Conclusions

The connectivity of real-time video counseling and telephone counseling for smoking cessation appears acceptable in regional and remote areas as an additional strategy for cancer prevention. This may inform the practices of quitlines.

PP26 Quitlink: Accessible Smoking Cessation Support for People Living with Severe and Enduring Mental Illness

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Background

People with severe mental illness (SMI) have a high, persistent smoking rate and are far more likely to die from smoking-related illnesses such as cancer than as a result of their psychiatric illness. Evidence-based smoking cessation interventions, such as quitlines, are underutilized by this group.

Aims

The primary aim is to examine the effectiveness of the Quitlink intervention on prolonged abstinence among smokers with SMI.

Methods

A multicenter prospective, randomized, open, blinded endpoint (PROBE) design will compare standard smoking care alone against Quitlink. A total of 382 smokers will be recruited from mental health services in Victoria, Australia.

Quitlink will utilize peer workers to refer smokers with SMI to Quitline, who will deliver a tailored and proactive smoking cessation intervention. The primary outcome measure will be 6 months prolonged abstinence. Repeated measures will be analyzed using generalized linear mixed models.

Results

We anticipate that for the primary outcome, success will occur in 1% of the control arm versus 8% in the intervention arm. Our qualitative component will identify potential improvements, and barriers to full participation and engagement with the service.

Conclusions

This is a highly translatable intervention resulting from linking two existing services (Quitline and mental health peer workers). It has the potential to greatly reduce a key cancer risk factor in people with severe mental illness.

Manuscript

PP27 Wingadhan Birrang (Woman's Journey) of Smoking Cessation During Pregnancy: Aboriginal and Torres Strait Islander Women Participating in the Indigenous Counselling and Nicotine (ICAN) QUIT in Pregnancy Pilot Study

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Background

Urgent calls have been made to reduce the rates of smoking during pregnancy among Aboriginal women. Supporting Aboriginal women to quit smoking during pregnancy is the most significant, potentially modifiable, risk factor for adverse infant and maternal health, and supports the long-term enhancement of Aboriginal, including circulatory disease, cancer, diabetes, kidney and

respiratory disease. Effective strategies for reducing smoking during pregnancy among Aboriginal women have not been found.

Aims

Describe the smoking behaviors and quit attempts among pregnant women participating in a pilot evaluation of a smoking cessation intervention.

Methods

Repeated cross-sectional surveys within a cluster randomised step-wedge trial. ICAN QUIT in Pregnancy pilot was implemented in six Aboriginal Community Controlled Health Services across NSW, SA and Qld. Participants were pregnant Aboriginal women, and expectant mothers of Aboriginal babies, <28 weeks gestation, >16 years old, smoking tobacco. Nicotine dependence measures, intentions to quit smoking, quit attempts, biochemically validated smoking abstinence, and use of NRT, were assessed at baseline, 4 and 12 weeks for all women participating, regardless of exposure to the intervention.

Results

In total, 22 women participated. At baseline 77% (17/22) reported likely to quit smoking, mean number of quit attempts 1.59 (SD 1.26). At 4 weeks, 80% (12/15) reported likely to quit smoking, mean number quit attempts 0.80 (SD 1.08), 27% (4/15) used NRT, one woman quit smoking. At 12 weeks, 92% (12/13) reported likely to quit smoking, mean number of quit attempts 1.67 (SD 1.72); 31% (5/16) reported using NRT, and three women (14%) quit smoking CO validated. No significant changes were noted for nicotine dependence measures.

Conclusions

Aboriginal pregnant women reported interest in quitting smoking, and made multiple quit attempts. None-the-less, only 14% quit smoking, highlighting that there might be a need for prolonged intensive cessation support, building on individuals experiences of previous quit attempts. Australian and New Zealand Clinical Trials Registry (Ref #ACTRN12616001603404)

PP28 Impact of Remoteness of Residence on Time to Diagnosis and Treatment of Head and Neck Cancer in NSW

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Background

More than 50% of head and neck cancers (HNCs) are diagnosed at advanced stage. Regional/remote HNC patients have poorer survival outcomes than their metropolitan counterparts, and are less likely to utilize radiotherapy for treatment. Patient and health-system factors may hinder early cancer diagnosis, reflected in longer time intervals along the pathway to treatment.

Aims

The aim of this study was to examine geographical variations in pathways to diagnosis and treatment for patients with HNC in NSW, and implications for survival.

Methods

Patients diagnosed with squamous cell carcinoma (SCC) of the oral cavity, oropharynx or skin of the head and neck from July 1, 2008 to June 30, 2013 were identified from an audit at the Mid North Coast Cancer Institute and the Royal Prince Alfred Hospital in Sydney. Study data were retrospectively collected from medical records and a dedicated HNC database.

Results

A total of 471 patients were eligible (78% male), mean age was 68 years and 59% had advanced stage HNC. At diagnosis, 40% and 60% lived in metropolitan and regional/remote NSW, respectively. The median total interval (symptom onset to treatment) was longer among for regional/remote patients (4.3 vs. 3.5 months, $P = 0.011$). While the treatment interval (time from

diagnosis to first treatment) was similar among regional/remote and metropolitan patients (median 34 vs. 32 days, $P = 0.69$), among 210 patients requiring post-operative radiotherapy, regional/remote patients had a significantly longer interval from surgery to adjuvant radiotherapy (median 58 and 42 days, $P < 0.001$). Regional/remote patients had poorer overall survival compared to metropolitan patients ($P = 0.003$), however there was no difference in cause-specific survival ($P = 0.46$).

Conclusions

Regional/remote HNC patients experience longer time to diagnosis and treatment than metropolitan patients, and have poorer survival outcomes. Understanding patient and health-system factors that facilitate and impede pathways to treatment can improve timely HNC diagnosis and treatment in regional/remote NSW.

PP29 Systematic Review of Interventions to Improve Health Providers Smoking Cessation Care in Pregnancy

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Background

Smoking in pregnancy is the most important preventable risk factor for poor maternal and foetal health outcomes. Health providers (HPs) report that they seldom perform all the recommended care elements (Ask about smoking, Advise to quit, Assess dependence, Assist cessation, Arrange Follow-up). Interventions to improve HPs provision of care include different components such as training, audit and feedback, and reminders.

Aims

To systematically review all available studies on interventions to improve HPs provision of smoking cessation care during pregnancy.

Methods

Five databases were searched. Screening of abstracts and full texts were conducted by two reviewers independently. Interventions were characterized according to the EPOC taxonomy. Random-effects meta-analyses examined intervention effects on the recommended care elements, and patient cessation rates. Estimates were either number of participants reporting each outcome, or mean score, transformed into Cohen's *d* to allow pooling of studies. Crude meta-regressions, and meta-analysis subgrouping, were performed to examine whether intervention effects on 'Ask', 'Advise' and 'Assist' could be explained by different intervention components.

Results

Out of 3165 manuscripts screened, 16 were included. Pooled intervention effects for each care component were: "Ask" $d = 0.47$, 95%CI 0.13, 0.81; "Advise" $d = 0.46$, 95% CI 0.02, 0.9; "Assess" $d = 0.98$, 95% CI 0.5, 1.45; "Assist" $d = 0.65$, 95% CI 0.46, 0.83; and "Arrange" $d = 0.84$, 95% CI 0.4, 1.29. Patient smoking abstinence rates showed a nonsignificant treatment effect ($d = 0.17$, 95% CI -0.04 , 0.38). Having a theoretical basis and inclusion of audit and feedback may improve intervention effectiveness for 'Ask', 'Advise' and 'Assist'.

Conclusions

Interventions designed to improve provision of smoking cessation care during pregnancy show a modest increase in all care elements, and may have the potential to improve cessation rates. Audit and feedback and basing the intervention design on a behavioral theory may improve effectiveness. Future research needs to focus on understanding which intervention components may further strengthen intervention effects.

PP30 Investigations of an EPID Based 3D Dose Reconstruction Method for Applications in MRI-Linac Radiotherapy

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Background

One of the challenges in radiotherapy with conventional linear accelerators (linacs) is that tumors tend to move during treatments. Using a Magnetic resonance imaging (MRI) device combined with a Linac (MRI-Linac) yields better soft tissue contrast images of the patients anatomy both prior to and during treatment, and could provide real-time tracking of tumors and better adaptive treatment planning. MRI-Linac systems will require dose verification methods to accurately validate the dose delivered to the patient.

Aims

To adapt a predictive fluence model and use this with images from an Electronic Portal Imaging Device (EPID) to reconstruct dose delivered to the patient on the Australian MRI-Linac system.

Methods

We utilize a fluence model that predicts the EPID dose, as well as a patient dose reconstruction (PDR) algorithm that back-projects this dose to the patient plane and calculates the three-dimensional patient dose via a collapsed cone convolution method. We will adapt the model and algorithm to calculate the patient dose for the Australian MRI-Linac system, accounting for the various differences in the system compared to conventional linacs.

Results

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The PDR algorithm has been demonstrated to work on a conventional TrueBeam linac using some simple square field plans as well as a Brain IMRT plan. A preliminary model for the Australian MRI-Linac has been made and is in the process of being optimized and tested. This optimization will include any adaptations to the algorithm to account for differences in the system (magnetic field, horizontal linac orientation, increased distance to EPID detector, etc).

Conclusions

MRI guided radiotherapy has the potential to greatly improve image guided radiotherapy for cancer patients. Our method will be able to reconstruct and verify that the dose delivered to the patient during treatment on the Australian MRI-Linac system is accurate.

PP31 Targeting the Tumor Suppressor PP2A-B55 α as a Therapeutic Strategy for Poor Prognosis Breast Cancer.

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Background

Breast cancer is characterized by the deregulation of multiple cellular signaling pathways (e.g. RAS/MAPK, PI3K/Akt, homologous recombination repair), which in normal cells are tightly regulated by protein kinases and phosphatases. The tumor suppressor serine/threonine phosphatase PP2A is a negative regulator of these pathways. The PPP2R2A gene, encoding the PP2A regulatory subunit PP2A-B55 α is commonly deleted in breast tumors, and low PP2A-B55 α associates with aggressive, poor prognosis breast tumors. However, the functional role of PP2A-B55 α loss in breast cancer is not known.

Aims

To investigate the functional role of PP2A-B55 α in breast tumorigenesis.

Methods

The effect of shRNA-mediated knockdown of PP2A-B55 α in normal mammary epithelial MCF10A cells and MCF7 breast cancer cells was examined using standard and 3D cultures. The effect of PP2A activating drugs in a panel of breast cancer cell lines was examined using cytotoxicity assays, and in an orthotopic xenograft mouse model of aggressive breast cancer (MDA-MB-231). A novel constitutive PP2A-B55 α knockout mouse was generated and crossed with MMTV-Neu transgenic mice to characterize the effect of reduced PP2A-B55 α on mammary gland development/tumor formation.

Results

PP2A-B55 α knockdown induced a tumorigenic phenotype in MCF10A 3D cultures, and increased proliferation and colony formation in MCF7 cells, associated with enhanced Akt and ERK signaling. Constitutive homozygous

knockout of PP2A-B55 α was lethal post embryonic day 18.5, with evidence of epidermal/neural tube defects. Viable, adult heterozygous mice displayed decreased mammary gland branching. Analysis of breast tumor onset/growth in MMTV-Neu mice with heterozygous PP2A-B55 α expression is ongoing. Treatment with PP2A activating drugs significantly inhibited breast tumor growth and metastases in human breast cancer cell lines in vitro and in vivo.

Conclusions

We show that PP2A-B55 α is essential for mammalian development and functionally important as a breast tumor suppressor. We further showed that pharmacological activation of PP2A is a potential therapeutic strategy for poor prognosis breast cancer patients.

PP32 Knowledge-Based Planning as a Real Time Review Quality Assurance Feedback Tool in the TROG 1501 SPARK Trial

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Background

Quality assurance (QA) in radiotherapy (RT) clinical trials is essential to ensure protocol compliance, patient safety and trial quality. However, protocol compliance does not necessarily ensure optimal plan generation.

Aims

This study aimed to demonstrate the feasibility and impact of Knowledge-Based Planning (KBP) feedback as part of the Real Time Review (RTR) process for the TROG 1501 SPARK trial (Stereotactic Prostate Adaptive RT Utilizing Kilovoltage Intrafraction Monitoring).

Methods

A knowledge based dose-volume histogram (DVH) estimation model and automated planning routine were created using 34 SPARK RT plans that had previously been submitted as part of the TROG QA program. The KBP routine was applied to 6 subsequent patients pre-treatment. A feedback report comparing the KBP generated DVH versus the initial plan was collated using a customized script and sent to the site within 24 h. Centers were asked to review the report and decide whether they would amend their clinical plan.

Results

Of the six patients, five were protocol compliant and one case was replanned due to a major protocol deviation. As a result of KBP feedback two of five (40%) cases, which were originally protocol compliant, were nevertheless replanned. Protocol dose constraints for all six cases were calculated and an average for each metric was generated. The mean dose-volume metrics were then compared between the initial submission, resubmission and KBP generated plans. Overall, the rectum, bladder, penile bulb and urethra planning risk

volume (PRV) demonstrated that an improved dose–volume relationship could be achieved compared to the initial submission and was implemented in practice for the three resubmitted cases. Variable results were observed for the femoral heads, demonstrating a potential dose trade off (Table 1).

Conclusions

KBP feedback was successfully incorporated into the RTR process for the SPARK trial and demonstrated that both improvements to and validation of plan quality for OAR dosimetry could be achieved. Further prospective investigation of the role of KBP in TROG clinical trials is planned.

PP33 Using Genomics and Pharmacokinetics to Predict 5FU Toxicity

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Background

Anticancer drugs have a narrow therapeutic index and wide interpatient variability that can lead to unexplained and unpredictable toxic effects. Pharmacokinetic (PK) testing has revealed that 30% of patients experience severe toxicity during treatment with 5-fluorouracil (5FU). Genomic analysis, particularly for drug-metabolizing enzymes (DMEs), may be a cost effective alternative to identify patients at risk of severe toxicity and allow for guided personalised drug dosing. We have previously completed a PK-study in 72 patients given adjuvant 5FU and leucovorin for early colorectal cancer. Main toxicities were neutropenia grade 3-4, mucositis grade 3-4 and diarrhea grade 2–4.

Aims

The aim of the study was to investigate SNPs in genes coding for enzymes proven or thought to be involved in 5FU metabolism, including DPYD and HAPB3, to develop prediction models for toxicity and cancer outcomes.

Methods

All samples (72 from the previous study and 24 new) were hybridized to Illumina Global Screening Array-24 chips, run on the Illumina HTS Assay platform and analyzed with GenomeStudio 2.0 system. SNPs will be investigated for correlation with 5FU PK.

Results

All DNA samples produced adequate call rates, although analytical quality was far greater for newer samples. Preliminary analysis indicates that compared to HapMap controls, patient samples display a higher rate of homozygous variant genotype of DPYD, a genotype leading to an inactive enzyme known to cause 5FU toxicity. Further analysis will correlate these results with observed 5FU PK phenotypes in the samples.

Conclusions

Illumina platforms can be used to identify SNPs in 5FU DMEs. This technology has potential, although its ability to predict 5FU toxicity routinely will require validation and sufficient clinical interest to make it viable. Until then routine PK analysis is the best method to facilitate 5FU dose personalization for patients with CRC and other cancers.

PP34 Neurotrophic Growth Factors and Their Receptors as Novel Therapeutic Targets in Esophageal Cancer

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Background

Esophageal cancer is one of the deadliest and most poorly understood cancers worldwide. Recent developments in oncology show that the family of proteins known as neurotrophin receptors and their ligand nerve growth factor are potential novel treatment targets, but their clinicopathological significance in esophageal cancer is unknown.

Aims

The aim of this study was to investigate the clinicopathological significance of neurotrophins NGF and proNGF and their putative receptors TrkA, P75NTR and sortilin, as well as to evaluate their potential utility as therapeutic targets.

Methods

In the present study, immunohistochemical analysis of the neurotrophic ligands NGF and proNGF, neurotrophin tyrosine receptor kinase TrkA, and the common neurotrophin receptor p75NTR, was performed on a series of 303 esophageal cancers of different histological subtypes versus 137 normal esophageal tissues.

Results

Increased presence of NGF, TrkA and p75NTR was detected in cancer tissues compared to normal adjacent tissues, with a particular association with malignant cancer. Additionally, expression of all neurotrophic receptors and their ligands was increased in squamous cell carcinoma compared with the adenocarcinoma histological subtype ($P < 0.0001$). Nerves in the tumor microenvironment were positive for TrkA, sortilin, p75NTR, proNGF, but not NGF.

Conclusions

These preliminary data suggest a preferential therapeutic value of targeting neurotrophic-signaling pathways in malignant squamous cell carcinomas of the esophagus.

PP35 The Neurotrophic Tyrosine Kinase Receptor TrkA and Its Ligand NGF are Increased in Squamous Cell Carcinomas of the Lung.

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Background

The neurotrophic tyrosine kinase receptor TrkA (NTRK1) and its ligand nerve growth factor (NGF) are emerging promoters of tumor progression. In lung cancer, drugs targeting TrkA are in clinical trials, but the clinicopathological significance of TrkA and NGF, as well as that of the precursor proNGF, the neurotrophin co-receptor p75NTR and the proneurotrophin co-receptor sortilin, remains unclear.

Aims

This study aims to detect the clinicopathological significance of TrkA and NGF, as well as that of the precursor proNGF, the neurotrophin co-receptor p75NTR and the proneurotrophin co-receptor sortilin.

Methods

Analysis of TrkA, NGF, proNGF, p75NTR and sortilin was conducted by immunohistochemistry and digital quantification in a series of 204 lung cancers of different histological subtypes versus 121 normal lung tissues.

Results

TrkA immunoreactivity was increased in squamous cell carcinoma compared with benign and other malignant lung cancer histological subtypes. NGF and proNGF were also increased in squamous cell carcinoma, as well as in

adenocarcinoma. P75NTR was increased across all lung cancer subtypes compared to normal lung. Sortilin expression was higher in adenocarcinoma and small cell carcinoma ($P < 0.0001$). Nerves in the tumor microenvironment were negative for TrkA, NGF, proNGF, p75NTR and sortilin.

Conclusions

This concomitant increased expression of both TrkA and NGF is suggestive of a NGF-mediated autocrine stimulation and suggests a preferential therapeutic value of targeting NGF-TrkA axis of squamous cell carcinoma of lung.

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