KEY

enade Presenter

PRESENTER’S INDEX

To be found at the end of this document, after the abstract listings.
PL1

Progression of Colorectal Cancer can be Initiated through Chromosomal Catastrophes and Punctuated Evolution

T Graham1; N Wright2; O W Cross1; I Tomlinson3; S Leedham3; V Mustonen1

1Barts Cancer Institute, London, UK; 2Oxford University Human Genome Center, Oxford, UK; 3Sanger Institute, Cambridge, UK

Purpose of the study: The precise initiator events of colorectal carcinogenesis remain elusive. Here we propose a punctuated evolutionary model whereby normal colon can acquire several copy number aberrations simultaneously and initiate carcinogenesis. Contrasting this process to growth of adenomas implies these chromosomal catastrophes as potential agents of the adenoma-carcinoma sequence.

Methods: We performed an in-depth evolutionary analysis of 11 cancer and 6 colon adenoma genomes through multi-region sequencing of primary site tissues. Using a specialist bioinformatics pipeline incorporating sub-clonal de-mixing of copy number variations (CNVs) we were able to discern ancestral states in each cancer or adenoma and observe the evolution of mutagenic processes through mutational signatures. Through implementation of novel mathematics we reconstructed the order of SNV and CNV acquisition therefore unveiling the precise initiator events of colorectal cancer in previously unseen detail.

Summary of results: Most of the well-described driver single nucleotide mutations seem to be attained and positively selected at the pre-malignant stage. In adenomas, the spoils of selection are still observable through greater frequency of subclonal drivers and proportionally greater diversity. A pivotal event in carcinogenesis seems to be the acquisition of several CNVs in cell division catastrophe. Though there is heterogeneity in the number and identity of chromosomes involved in this process many, such as 1p/1q, 5q, and 11p that have been previously observed in cancer, are present.

Conclusions: Certain colon cancers seem to be initiated by several synchronous chromosomal alterations that we term a chromosomal catastrophe. Evidence suggests that the traditional view of CRC as a point mutation driven disease, centered on disruption of Wnt signaling may be an over-simplification and the focus of evolutionary analysis on point mutations needs to shift to include CNV alterations.

PL2

Clusters of Nuclear Beta-Catenin Accumulating Cells Form Secretory Hubs in Adamantinomatous Craniopharyngioma

JR Apps1; N Jani1; JM Gonzalez-Meljem1; K Tossell1; T Carroll1; MA Ungless1; J Gill3; H Williams1; TS Jacques1; JP Martinez-Barbera1

1Institute of Child Health, UCL, London, UK; 2MRC Clinical Sciences Centre, Imperial, London, UK

The majority of Adamantinomatous Craniopharyngiomas (ACPs) contain activating mutations in CTNNB1, however immunohistochemistry demonstrates that nuclear-cyttoplasmic accumulation of β-catenin only occurs in a small proportion of cells, either individually throughout the tumour or more frequently grouped in clusters. Mouse models indicate that these clusters initiate tumourigenesis in a non-cell autonomous manner. Murine expression arrays and immuno-staining (murine and human) have shown that the clusters have a senescent and secretory phenotype, exhibiting markers of cellular senescence (p16, p21, SAHF), and express some SASP factors.

We assessed several senescence features in BCs. This included: cellular senescence-associated β-galactosidase (SA-β-gal) activity and chromatin remodeling through histone deacetylation. This study examines human clusters expression profile in an unbiased manner using RNA sequencing.

Results: Laser capture micro-dissection was performed on 2 human ACP cases to isolate cluster and non-cluster tumour cells. RNA was extracted, amplified and sequenced. Differential expression analysis was performed and the expression pattern assessed using gene set enrichment analysis (GSEA). In addition, previously published expression array from murine non-cancerous clusters were used with the human results.

646 genes were identified as up-regulated in human clusters (adjusted p-value<0.01) compared to non-cluster tumour cells. As expected, and similar to murine clusters, these include target genes of the WNT/β-catenin pathway and secretory molecules (e.g. SHH, FGF3). GSEA revealed enrichment of senescence genes, confirming previous targeted assays. Clustering of murine expression data with those genes differentially expressed in the human revealed murine and human clusters group together.

These results support the current hypothesis from murine experiments that clusters are acting as a secretory hub. Targeting the pathways activated by such signalling offers an attractive therapeutic opportunity, for which the results suggest the mouse model is well placed for testing.

PL3

Micro-CT of Postmortem Fetal Hearts: A Comparison of Imaging and Macroscopic Dissection

JC Hutchinson1; OJ Arthurs1; MT Ashworth1; AT Ramsey1; W Mifsud3; CM Lombardi1; NJ Sebire1

1Great Ormond Street Hospital, London, UK; 3Sanger Institute, Cambridge, UK

Congenital heart disease is common and may lead to a decision for termination of pregnancy. Factors such as size and macrocaren can make accurate diagnosis of congenital heart disease challenging at fetal autopsy, especially following fetocide. Post-mortem MRI is useful in guiding pathological dissections; however, accuracy falls in specimens of less than 500g. Micro-CT can provide high resolution images (up to 3 micrometers) but has not been systematically compared with fetal autopsy for diagnostic accuracy in humans. We hypothesised that micro-CT could provide useful diagnostic information in fetal congenital heart disease and compared it with the gold standard of autopsy.

Six ex-vivo fetal hearts (17 - 23 weeks gestation; weight 1.1g - 5.3g) underwent micro-CT examination as part of an ethically approved study with full research consent in every case. 21 indices were evaluated for each dataset at both micro-CT and autopsy in a single-blinded fashion. All micro-CT scans were diagnostic, with excellent internal contrast. The correct overall diagnosis was made from micro-CT data in all cases. Agreement between micro-CT imaging and dissection was demonstrated for 114/126 (overall concordance of 95.8% (95%CI: 90.5, 98.2). Micro-CT evaluation had a preliminary sensitivity of 83.2% (95%CI: 67.5, 94.1) and specificity of 98.9% (95%CI: 94.1, 99.6). A particular advantage of micro-CT was when evaluating the macrocardium of macerated hearts; this addresses an important diagnostic challenge for pathologists. Micro-CT provides accurate 3D volumes of complex congenital fetal heart disease.

This approach potentially represents a significant advance in post-mortem imaging and confirms the potential of this technology for non-invasive examination of small fetuses and organs. Centres lacking specialist paediatric cardiac pathology input may especially benefit. Future studies should expand to include in-vivo postmortem evaluation and double-blinded comparison.

PL4

A Role for Senescence in Childhood Epilepsy: Evidence from Balloon Cells in Paediatric Focal Cortical Dysplasia

F Scerif1; A Alahdal1; G Anderson2; E Zabala Aldunate1; M Tisdall2; JH Cross1; JP Martinez-Barbera1; TS Jacques1

1UCL Institute of Child Health, London, UK; 2Great Ormond Street Hospital, London, UK

Introduction: Focal cortical dysplasia (FCD) is a malformation of cortical development, is a frequent cause of multidrug resistant paediatric epilepsy. FCD type Ib is characterised by a population of unique abnormal cells known as balloon cells (BCs).

We provide evidence that BCs are in senescence. Cellular senescence is a state of irreversible cell cycle arrest. Like BCs, senescent cells often have a flat, enlarged, multinucleated morphology. Other features of senescence include activation of p53/p21 and/or p16 tumour suppressor pathways, an enhanced DNA damage response (DDR), the senescence-associated secretory phenotype (SASP), increased senescence-associated β-galactosidase (SA-β-gal) activity and chromatin remodeling through senescence-associated heterochromatin foci (SAHF) formation.

Methods: We assessed several senescence features in BCs. This included: cellular proliferation, the expression of cell cycle inhibitors and DDR proteins, the SASP, SA-β-gal, and the presence of SAHF. Appropriate immunohistochemical and histochemical techniques were used on surgical samples from patients with FCDIb as well as controls.

Results: BCs were positive expressed markers cell cycle inhibitors (p16, p21, p53) and DDR (pH2AX, ATM). Histochemical staining of SA-β-gal showed clear positivity in BCs. BCs were negative for the proliferative marker Ki67. BCs were also found to contain SAHF, and express some SASP factors.

Conclusions: We have shown BCs, a key pathological cell of FCDIb, to be positive for various well-established markers of cellular senescence. Further investigations are required to study signalling pathways associated with BCs in senescence and to define the interactions between the different pathways. A better understanding of these signaling pathways may lead to the identification of novel therapeutic targets.
PL5

Tubal Ligation and Ovarian Cancer Risk in a Large Cohort: Substantial Variation by Histological Type

PK Gaitskell; J Green; K Pirie; G Reeves; V Beral; MWS Collaborators
Cancer Epidemiology Unit, University of Oxford, Oxford, UK

Histopathological and molecular studies suggest that the different histological types of ovarian cancer have distinct origins. Tubal ligation (sterilization) has been associated with a reduced risk of ovarian cancer, but few epidemiological studies have been large enough to reliably explore possible variation by tumour histotype.

We investigated whether a woman’s risk of ovarian cancer is associated with prior tubal ligation, and whether this differs for the four main histotypes (serous, mucinous, endometrioid and clear cell), in a prospective study of UK women. Participants were recruited in 1996-2001, and completed a detailed questionnaire. Follow-up was via routine data from central cancer registries. Using a Cox proportional hazards model, we estimated adjusted relative risks (RR) of ovarian cancer associated with tubal ligation. The study population included 1.1 million women, aged 56 years on average at recruitment; 8,035 ovarian cancers accrued during mean follow-up of 13.8 years.

Overall, women with tubal ligation had a 20% reduction in risk of ovarian cancer (RR: 0.80, 95% CI: 0.76-0.85), but there was substantial heterogeneity in risk by histotype (heterogeneity: p=0.0001). For serous tumours, the most common histotype (n=3,515), risks differed significantly between high-grade (RR: 0.77, 95% CI: 0.67-0.89) and low-grade tumours (RR: 1.13, 95% CI: 0.89-1.42); heterogeneity: p=0.007. Relative risks were almost halved for endometrioid (n=690, RR: 0.54, 95% CI: 0.43-0.69) and clear cell tumours (n=401, RR: 0.55, 95% CI: 0.39-0.77), but there was no association between tubal ligation and mucinous tumours (n=836, RR: 0.99, 95% CI: 0.84-1.18).

The significant differences by tumour histotype are unlikely to be due to confounding and are consistent with hypotheses that high-grade and low-grade serous tumours have different origins, and that some endometrioid and clear cell tumours might arise from cells and/or carcinogens travelling through the Fallopian tubes.

PL6

Molecular Interactions of Polo-like Kinase 1 (PLK1) in Colorectal Cancer

WTW Ng; J Shin; T Yang; T Roberts; B Wang; J Begg; CS Lee
1Discipline of Pathology, School of Medicine, Western Sydney University, Sydney, Australia; 2Discipline of Medical Oncology, School of Medicine, Western Sydney University, Sydney, Australia; 3South Western Sydney Clinical School, University of New South Wales, Sydney, Australia; 4Department of Medical Physics, Liverpool and Macarthur Cancer Therapy Centre and Ingham Institute for Applied Medical Research, Sydney, Australia

Radiotherapy (RT) improves the local control of rectal cancer but patient responses to RT are variable. Biomarkers are needed to determine the radiosensitivity of these tumours. Microsatellite instability (MSI) accounts for 15% of colorectal cancer (CRC) cases and the associated proteins were shown to be involved in DNA damage responses (DDR). PLK1 promotes cell cycle progression and is also involved in recovery from DNA damage. Dereactivation of PLK1 causes genetic instability due to G2-M checkpoint escape. PLK1 overexpression is associated with poor tumour prognosis.

This study investigated the interactions between PLK1, MSI and ionising irradiation (IR) and explored the molecular mechanisms that are associated to PLK1 deregulation in CRC. siRNA was used to deplete PLK1. The effects of the knockdown and IR were then analysed by real-time quantitative PCR, western blot, cell survival assays, caspase 3/7 assay, annexin V binding assay and cell cycle analysis. Sanger sequencing was performed to detect mutations in PLK1 gene. In MSI high cells (HCT116, SW48), PLK1 expression decreased post-IR whereas it remained unaffected in microsatellite stable (MSS) cells (Colo320DM, T84). MSI high cells were more radiosensitive than the MSS cells. PLK1 reduction resulted in substantial reduction of cell survival, as well as increased induction of apoptosis and G2/M blockage in Colo320DM, HCT116 and SW48 cells but not T84 cells. These effects were additive in the responsive cells when the treatment was combined with IR. PLK1 sequencing studies showed that only secondary mutations were detected at the silencer region of PLK1 in HCT116 and SW48. In conclusion, MSI has an impact on PLK1 expression which in turns affects the cell survival after IR. Moreover, PLK1 knockdownd additionaly improved the effects of IR in some of the CRC cells, including some radioreistant cells. Lastly, sequencing concluded that mutation did not play a major role in PLK1 expression level in CRC.