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KEY

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PL1

Characterisation of a Novel *USP9X-DDX3X* Fusion in Acute Lymphoblastic Leukaemia and its Functional Significance

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Background: Acute lymphoblastic leukaemia (ALL) is a haematological malignancy derived from lymphoid progenitor cells. It causes 25% of all cancers in patients under the age of 15. B-cell ALL (BCP-ALL) represents 75% of ALL cases. ALL risk stratification, which takes into account frequent and well-characterised genomic aberrations, drives treatment assignment. Investigation of newly-identified recurrent aberrations leads to improved treatment assignment, development of new therapies, and reductions in over- and undertreatment. A novel *USP9X-DDX3X* fusion, removing the *USP9X* ubiquitin-specific protease domain and the *DDX3X* promoter, was recently discovered at high rates in two ALL subtypes. Both genes have roles in cancer, but little is known about their activity in ALL.
Aim: Begin functional characterisation of *USP9X* and *DDX3X* in ALL.
Methods: *USP9X* and *DDX3X* were knocked down separately using lentiviral shRNA in human BCP-ALL cell lines REH and NALM-6. Cell survival was monitored over 7 days. Knockdowns were confirmed by western blot.
Results: *USP9X* knockdown reduced cell survival by day 4 in REH ($p<0.01$, 3 replicates). *DDX3X* knockdown reduced cell survival by day 4 in both REH ($p<0.01$, 3 replicates) and NALM-6 ($p<0.05$, 3 replicates). Prior data showed a reduction in cell survival by day 4 after *USP9X* knockdown in NALM-6 ($p<0.01$, single experiment).
Conclusion: *USP9X* and *DDX3X* knockdowns independently reduce cell survival in two BCP-ALL cell lines. Therefore, both genes represent potential therapeutic targets in BCP-ALL subtypes. Mechanistic elucidation of the effect on proliferation will facilitate up- or downstream targeting. Further work is required to confirm these results and allow their extrapolation to BCP-ALL more broadly.
Acknowledgements: Thanks to *The Pathological Society of Great Britain and Ireland* for funding this research.

PL2

Alterations of Pericytes in the Bone Marrow Stem Cell Niche of Patients with Type 2 Diabetes Mellitus

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Background: Type 2 Diabetes Mellitus (T2D) endangers vascular cell integrity and remodels the bone marrow (BM) with microangiopathy and hematopoietic dysfunction. Pericytes are mural cells that physically and molecularly support the microvasculature but are lost in diabetic retinopathy. Little is known about pericyte alterations in the T2D BM, where they can be identified as CD146$^+$ cells. This study analyses the expressional and functional changes of CD146$^+$ BM pericytes in T2D.
Methods: CD34$^-$CD45$^-$CD146$^+$ (CD146$^+$) cells from non-T2D and T2D human BM were expanded in vitro. They were characterized for mesenchymal, hematopoietic, and endothelial markers by flow cytometry and immunocytochemistry. Functional assays of proliferation, viability, and apoptosis were performed. Angiogenic factors were assessed by qPCR and ELISA. Phosphorylated Akt (p-Akt) S473 and T308 were measured by western blotting.
Results: CD146$^+$ non-T2D and T2D cells were similarly positive for CD146 and mesenchymal markers CD105, CD73, and CD90. They were positive for nestin, leptin receptor (Lep-R), and NG2. They lacked expression of hematopoietic (CD34, CD45) and endothelial markers (vWF). Platelet-derived growth factor receptor-β (PDGFR-β) was less expressed in T2D cells. Both non-T2D and T2D passage 0 cells had a stellate morphology at confluency. T2D CD146$^+$ cells had a significantly reduced proliferation, viability, and increased apoptosis. T2D cells had an upregulation of angiopoietin-1 and angiopoietin-2, but a downregulation of most angiogenic factors compared to non-T2D cells, indicating diminished angiogenic potential. P-Akt S473 and T308 were reduced in T2D cells.
Conclusion: BM CD146$^+$ pericytes are functionally impaired in T2D and this may be due to compromised Akt signalling. This could relate to the BM microangiopathy and delayed healing that occurs in T2D patients.
This work was supported by the Pathological Society Intercalated Degree grant.

PL3

The use of Affymetrix OncoScan to Validate Biomarker Screening Results in Patients Enrolled in the MRC FOCUS4 Trial: The Quest to Get More, from Less

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A personalised medicine approach to cancer treatment, means larger, more detailed biomarker data is required from small and/or challenging clinical samples. The Affymetrix OncoScan FFPE Assay combines mutation screening with genomic copy number (CNV) and loss of heterozygosity (LOH) analysis. We report results from 78 MRC FOCUS4 clinical trial patients, on which prospective biomarker screening was previously carried out using pyrosequencing and immunohistochemistry (IHC).
One sample failed initial OncoScan QC metrics and was not analysed further. There was (70/77) 90.9% concordance between pyrosequencing and OncoScan mutation screening. Mutations detected in three samples by pyrosequencing, were not included in the OncoScan panel. Two low level mutations were 'missed' by OncoScan, and two additional mutations were detected on OncoScan, but with low QC metrics. TP53 mutations were detected in 19/77 (24.7%) of samples. Of these, four also contained a BRAF mutation, three also contained a KRAS mutation and one contained both a KRAS and PIK3CA mutation. Of the eight pTEN negative samples, 25% contained pTEN mutations, 62.5% contained RAS mutations and one also contained a TP53 mutation. Samples were grouped by mutational status, in relation to how patients would be randomised into one of the FOCUS4 trial arms, and examined for CNV. All groups showed changes common to colorectal cancer: losses in 8p, 17p, 18q and gains in 7p, 8q, 20q. There were no changes unique to, or significantly more common in any subset of samples. A problem when selecting patient treatment, on the basis of a single mutation, is the presence of other mutations within a tumour, which may ultimately drive resistance, through an alternative pathway. These additional mutations may contribute to the heterogeneous responses seen by patients, to identical therapies. Suitable technologies should be employed to capture as much molecular information as possible, to fully inform treatment selection.

PL4

Colon Cancer: Computer Assisted Quantitative Immunohistochemical Analysis Improves RNA Expression Based Prognostic Score

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We compared the power and utility of quantitative computer assisted analysis of standard immunohistochemical markers with RNA expression analysis as prognostic indicators in a well characterized series of patients with colo-rectalcancer (CRC). Tissue micro-array slides from 625 CRC patients were immunostained for CD3, CD4, CD8, CD20, FOXP3, and scanned using a Panoramic Digital Slide Scanner 250 Flash II (3DHistech). Computer assisted quantitative analysis was then performed using a custom script in Cognition Network Language (Definiens®). Results of this analysis were compared with RNA expression data obtained from the same series of samples using Affymetrix technology and respective contributions to a prognostic score were determined. RNA expression data showed a statistically significant correlation with IHC marker expression, the latter yielding more robust results for weakly expressed transcripts. Data from both analyses were integrated with survival data and their contributions to a prognostic score compared. An optimal model was developed combining IHC data for CD8 and FOXP3 with RNA expression for CD3 and CD4 and was shown to be independently significant on multivariate analysis.
Computer assisted quantification of IHC staining is a powerful method that can improve on RNA expression based prognostic scores and can be applied in high throughput settings.

PL5

Expression of Mel-CAM and HSD3B1 in Cervical Carcinoma

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Trophoblastic tumours as placental site trophoblastic tumour (PSTT) and epithelioid trophoblastic tumour (ETT) show morphological overlap with cervical squamous cell carcinoma (SCC). These trophoblastic tumours can invade the cervix and in some cases there may be difficulty in differentiating them from cervical SCC based on morphological assessment alone. Immunohistochemistry (IHC) may be helpful in these cases using markers of trophoblastic differentiation which would be expected to be negative in SCC. This study investigated the expression of 2 trophoblastic markers- Mel-CAM and HSD3B1 in cervical carcinoma to assess their value in distinction between cervical carcinoma and trophoblastic tumours.
Forty-three cases of cervical cancer comprising 25 SCCs, 13 adenocarcinomas and 5 adenosquamous carcinomas as well as HeLa cell line were studied. Mel-CAM and HSD3B1 expression was examined in all 43 samples of cervical cancer using immunohistochemical staining. Mel-CAM and HSD3B1 expression was also studied in HeLa cells by IHC and Western blotting (WB).
Mel-CAM showed weak, moderate or strong membranous and rarely cytoplasmic expression in 18 cases (42%), including 14 SCCs (77.8%), 1 adenocarcinoma (5.6%) and 3 adenosquamous carcinomas (16.7%). HeLa cells showed strong membranous expression of Mel-CAM on IHC and a band at the correct molecular weight was identified on WB. HSD3B1 showed weak, moderate or strong cytoplasmic expression in 42 cases (98%), including 24 SCCs (57%), 13 adenocarcinomas (31%) and 5 adenosquamous carcinomas (12%). On the contrary, no expression of HSD3B1 was detected in HeLa cells by either IHC or WB.
This is the first study to investigate and demonstrate the expression of Mel-CAM and HSD3B1 in cervical carcinoma. The findings show that Mel-CAM and HSD3B1 are not exclusively expressed by trophoblastic tumours and hence cannot be unequivocally reliable in differentiating between trophoblastic and cervical tumours.

PL6

Role of Myc in Choroid Plexus Tumour Pathogenesis

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Introduction: Choroid plexus tumours (CPT) constitute 2-5% of all paediatric brain tumours. They can spread along the neuraxis and are known to recur after treatment. Understanding the molecular mechanisms underlying their formation will be important to devise more efficient therapeutic strategies. c-Myc is a proto-oncogene deregulated in various malignancies, including paediatric brain tumours. Here, we test the hypothesis that deregulation of c-Myc expression plays a role in human CPTs.
Methods: The brains of RosaMycIB12;Nestin Cre mice, overexpressing c-Myc in neural progenitor cells, were examined at 20 months of age. 42 human CPT samples obtained from the BRAIN UK Network and CCLG were tested for c-Myc expression and amplification by immunohistochemistry and fluorescence in-situ hybridisation. Publicly available gene expression microarrays [GEO GSE60886] for 40 CPT cases were screened for correlation with c-Myc probes using R software. Heatmap and pathway analysis was performed using DAVID Bioinformatics Resources and KEGG.
Results: CPT developed in 84% of transgenic mice with activated c-Myc construct. A total of 43% human CPT cases expressed c-Myc on IHC. None of the 16 C-MYC+ tumours examined showed amplification. From bioinformatics analysis of the published database, we identified 212 genes, the expression of which correlated with c-Myc expression. Among the canonical pathways associated with high c-Myc expression, were TNF and Cytokine-Cytokine receptor pathway. Furthermore average CD3+ T-lymphocyte infiltrate count/HPF in our cohort was 12.3 vs 3.8 ($p<0.05$) for c-Myc positive vs negative tumours.
Conclusions: Overexpression of c-Myc in neural progenitor cells leads to CPT development in a high proportion of the mutant mice. More than a third of the human CPT tested express c-Myc, although this was not caused by gene amplification. Analysis of published dataset suggests that c-Myc may influence CPT pathogenesis via inflammatory mediated pathways.

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