WHOLE-GENOME SEQUENCING OF NORMAL STEM CELLS PROVIDES NOVEL INSIGHTS INTO HUMAN NATIVE HEMATOPOIESIS AND LEUKAEMIA AEITOLOGY

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Introduction Mature blood and immune cells are produced by the process of hematopoiesis, which is orchestrated by self-renewing hematopoietic stem cells (HSCs) and multipotent progenitor cells (MPPs) in the bone marrow. As people age, clonal expansion of mutated stem cells within the bone marrow is more commonly observed, which is associated with increased risk of developing haematological malignancies. However, the processes underlying mutation accumulation in normal stem cells and the clonal composition of the blood tissue within the human bone marrow compartment remain unknown.

Material and methods We determined genome-wide mutation patterns in HSCs and downstream MPPs by sequencing clonally expanded primary cells derived from bone marrow of multiple human donors of different ages.

Results and discussions We found that base substitutions accumulate gradually during life at a rate of approximately 16 novel mutations per year, while insertions and deletions occur more sporadically and at low numbers. The number and types of mutations were similar between HSCs and MPPs, suggesting that differentiation and quiescence status does not affect somatic mutation load. The majority of the base substitutions in blood progenitors could be attributed to a novel mutational signature, which also explains the majority of base substitutions within the human bone marrow compartment.

Conclusion Our data demonstrate that developmental clones have differential contribution to the HSC and MPP compartments, and while these sub-clones exhibit multipotency, branches contributing predominantly to HSCs have an enriched presence in the megakaryocyte lineage. Together, our approach highlights novel features of human hematopoiesis and its implications for leukemogenesis.

Symposium: Microbiome and Microenvironment

LOSS OF P53 DRIVES SYSTEMIC NEUTROPHILIC INFLAMMATION IN BREAST CANCER

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Introduction Metastasis remains the primary cause of death for breast cancer patients. The metastatic cascade is largely regulated by interactions between cancer cells and their microenvironment. Using a genetically engineered mouse model (GEMM) for breast cancer, we have previously shown that mammary tumours elicit a systemic inflammatory cascade, involving γδ T cells and neutrophils, which promotes metastasis. Breast cancer is a heterogeneous disease, and it is unknown whether and how the genetic make-up of primary tumours influences pro-metastatic inflammation. Therefore, we set out to dissect the cancer cell-intrinsic genetic events that activate systemic inflammation in breast cancer.

Material and methods We make use of a panel of 16 GEMMs for breast cancer with different tissue-specific genetic modifications driving tumorigenesis, which recapitulate all subtypes of human breast cancer.

Results and discussions We observed increased levels of neutrophil-activating cytokines and elevated numbers of neutrophils in mice bearing p53-deficient mammary tumours, compared to mice with p53-proficient tumours. To identify the cancer cell-derived factors underlying enhanced systemic inflammation upon p53 loss, we compared the transcriptome profiles of p53-deficient with p53-proficient mammary tumours. Consistent with the enhanced neutrophil activation, we observed significant changes in various immune-related pathways in p53-deficient tumours. Using CRISPR/Cas9-based technologies in GEMM-derived cancer cell lines, we investigated the influence of candidate genes that are consistently up-regulated in Tp53+/− tumours on the cellular crosstalk in the tumour microenvironment.

Conclusion Together, these data reveal that cancer cell-intrinsic loss of p53 dictates the communication between cancer cells and their microenvironment, leading to activation of a systemic neutrophilic inflammatory cascade.

IMPACT OF COLIBACTIN-PRODUCING ESCHERICHIA COLI ON IMMUNE MICROENVIRONMENT IN PRECLINICAL COLORECTAL CANCER MODELS

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Introduction Increasing evidence link the immune microenvironment, microbiota and colorectal cancer (CRC). E. coli pkS are more frequently detected on CRC patient mucosa and exhibit procarcinogenic properties in CRC murine models. Aim of this work was to evaluate the impact of chronic infection by colibactin-producing E. coli on immune cells in APCmin mice.

Material and methods Min mice were per os inoculated with a CRC E. coli pkS strain (11 G5), non-pathogenic E. coli (K12) or PBS. Induction of oxidative stress and inflammation during the infection was evaluated using optical in vivo imaging (IVIS spectrum). After 7 weeks, number and volume of...
polyps were evaluated and colonic samples were histologically analysed. Immunofluorescent staining of immune cells (neutrophils and T cell populations) was performed to quantify positive cells in three interesting colon areas: mucosa, lymphoid follicle and tumour using an innovative algorithm created with Tissue Studio software. In parallel, quantification of immune cells (lymphoid populations) was performed in mesenteric lymph node (MLNs) by flow cytometry.

Results and discussions Using optical imaging, we detected a significant increase of oxidative stress and inflammation in the gut after thirty days of 11 G5 infection. However, using our specific algorithm and CD45 immunostaining, we observed a significant increase of lymphoid follicle size in the gut of mice infected with the 11 G5 strains. Interestingly, follicle size was positively correlated with tumour volume suggesting an association between pro-carcinogenic properties of 11 G5 strain and gut immune response. In addition, we observed an increase of neutrophils on the mucosa and lymphoid follicle of 11 G5 infected mice. These results can be linked to our in vivo optical imaging observations. Moreover we observed a trend to decrease of CD3⁺ T cells on mucosa and tumour for the 11 G5 group, suggesting a possible link between E. coli pks + and anti-tumour T cells. Finally we noticed a significant decrease of CD4⁺ CD25⁺ anti-inflammatory T cells in MLNs of 11 G5 infected mice, negatively correlated with gut bacteria colonisation.

Conclusion Here we report a potential immunomodulatory effect on gut microenvironment by pks+E. coli, which could be associated with its carcinogenic effect. Our results suggest that gut lymphoid follicle and immune cells such as neutrophils and T cells could be involved in this process. This work shows a link between immune microenvironment, pathogenic E. coli and tumour development.

Symposium: Systems Medicine – Making Sense of Big Data

Abstracts

33 PAN-CANCER WHOLE GENOME SEQUENCING REVEALS PATTERNS OF SUBLONAL MUTATIONS, SIGNATURE CHANGES AND SELECTION

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Introduction During their development, tumour cells accumulate somatic mutations, structural variants and copy number alterations (CNAs). Driver events facilitate clonal expansions and lead to intra-tumour heterogeneity (ITH). While ITH is an important therapeutic challenge, its degree among different cancer types is largely unknown.

Material and methods The pan-cancer analysis of whole genomes (PCAWG) enabled us to characterise ITH in an unprecedented set of 2778 tumour samples representing 36 histologically distinct cancer types. We applied six CNA callers and eleven subclonal reconstruction algorithms to integrate their solutions into robust consensus copy number profiles and subclonal reconstructions.

Results and discussions Our analysis revealed pervasive ITH in all examined cancer types. We found at least one subclone in 96.7% of the 1801 samples for which we had statistical power to detect subclones. In addition, we find that the average proportions of subclonal point mutations, indels, SVs and CNAs are highly variable across cancer types. These observations suggest distinct evolutionary narratives of each histological cancer type.

Analysis of dN/dS ratios shows clear signs of positive selection within both clonal and subclonal mutations. We also identified subclonal mutations in driver genes that are recurrently hit and we found a significant enrichment of subclonal mutations in genes responsible for chromatin regulation. More than 5% of tumours contain driver mutations in genes for which specific treatment is available only in subclones, indicating the importance of assessing the clonality of targeted mutations for clinical decisions.

Mutational signatures in the analysed samples show changes in activity over the course of tumour development. Characteristic carcinogen signatures, e.g. UV light exposure in melanomas, make less contributions to subclonal than clonal mutations, while APOBEC-induced mutagenesis has increased activity during the subclonal phase.

Conclusion The absence of a detectable driver mutation in a majority of subclones suggests that late tumour development is frequently driven by CNAs or genomic rearrangements, or that a significant number of late drivers have yet to be identified. We found that selection is widespread and likely the rule rather than the exception and we identified differential activity of mutational signatures, reflecting successive waves of subclonal expansion.

34 GENE FUSIONS IN 1,015 HUMAN CANCER CELL LINES: INTEGRATING LARGE-SCALE GENOMIC DATA, HIGH-THROUGHPUT DRUG AND CRISPR/CAS9 SCREENS TO ASSESS FUNCTIONAL RELEVANCE AND THERAPEUTIC POTENTIAL

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Introduction Translating our understanding of genetic alterations in cancer into clinical care remains a major challenge. The discovery of gene fusions such as EML4-ALK in lung cancer and BCR-ABL1 in chronic myeloid leukaemia have already led to changes in clinical care. Advances in next-generation sequencing have accelerated the rate at which novel gene fusions are discovered, but important questions remain about their roles in promoting oncogenic phenotypes and their relevance in drug response. Here, we combine RNA sequencing, CRISPR/Cas9 screens and high-throughput drug sensitivity data in a panel of 1000 human cancer cell lines to examine