PO-209 CRISPR/CAS9 BASED DEVELOPMENT OF RNAI RAT MODELS FOR DRUG DISCOVERY

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Introduction Genetically engineered mouse models have become the premier organism for dissecting cancer mechanisms and evaluating novel drug targets in vivo due to the availability of mouse embryonic stem cells that could be genetically manipulated in vitro. Despite the utility of mouse models, the rat has historically been the major model species in many biomedical fields, notably toxicology and carcinogenicity testing; and for many scientists, the rat still remains the preferred rodent due to their larger size for surgical manipulation, repeat blood sampling, and their cognitive and physiological characteristics that more closely resemble humans than their mouse counterparts. Now, with the advent of CRISPR/Cas9 technology, generation of genetically engineered rats is now a possibility.

Material and methods Here, we take advantage of our two-step engineering approach and exploit the efficiency of CRISPR-based targeting to develop RNA interference rat models that enable inducible and reversible gene silencing to simulate therapeutic regimes. When combined CRISPR-Cas9 gene modification, we can not only generate cancer de novo in a few weeks time, but also mimic drug therapy via RNAi in the same animal, giving us advanced capabilities to perform preclinical studies in vivo.

Results and discussions We demonstrate that our approach allows us to rapidly generate RNAi rat models and mimic the function of the targeted small molecule inhibitors, such as BET inhibitors targeting Brd4. We compare our results to our Brd4 RNAi mice and demonstrate organism variances that provide valuable insight to cross-species differences. These results demonstrate that our high-throughput system currently used to generate RNAi mouse is also applicable to the rat system and, by extension, other mammalian models.

Conclusion Inducible RNAi rat models will undoubtedly be powerful tools that can be used to model human cancers, to mimic the action of putative drugs, and to assess the potential of therapeutic targeting strategies in vivo prior to the costly drug development, ultimately guiding the development of safer and more effective drugs.

PO-208 HUMAN ZEBRAFISH XENOGRAPHS AS THERAPY SENSORS FOR BREAST CANCER

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Introduction Despite great advances in biomarker-driven therapies, we still lack methods to predict how a specific cancer in a unique patient will respond to a given therapy. This exposes some patients to unnecessary toxicities and delays access to other potentially effective therapies.

Material and methods Recently, we developed and optimised zebrafish-larvae-xenografts for personalised medicine. As a proof-of-principle, we screened the current standard of care for colorectal cancer (CRC), from 1 st to 3 rd lines of treatment, following the international cancer therapy guidelines. After showing a similar response to therapy between mouse and fish xenografts, we demonstrate the feasibility of generating zebrafish Patient Derived Xenografts (zPDX) and provide proof-of-concept experiments that compare response to therapy between patients and their matching zPDX (Fior et al, 2017).

Results and discussions We are now replicating the same technology for triple negative breast cancer (TNBC). Surprisingly, we found that TNBC cells can respond differently to compounds of the same family, such as taxanes (docetaxel vs paclitaxel) and anthracyclines (epirubicin vs doxorubicin). These findings may have clinical implications since nowadays they are considered equivalent. Moreover, we show that bevacizumab, an anti-VEGF therapy without any biomarker available and contradicting results in the clinic, can be screened in zebrafish xenografts.

Conclusion We are generating breast zPDX and comparing response to therapy in patients to their matching zPDX both in early and advanced disease. Altogether, our preliminary results suggest that zebrafish-xenografts constitute a promising in vivo assay for screening chemotherapy in breast cancer.
Introduction The lung is a highly oxidative environment, tolerated through the engagement of tightly controlled stress response pathways. A critical stress response mediator is the transcription factor Nuclear Factor Erythroid-2-Related Factor 2 (NFE2L2/NRF2), which is negatively regulated by Kelch-like ECH-Associated Protein 1 (KEAP1). Alterations in the KEAP1/NRF2 pathway have been identified in 23% of lung adenocarcinomas, suggesting that deregulation of the pathway is a major driver in lung cancer.

Material and methods We conditionally deleted Keap1 (Keap1f/f) in the lung, utilising inhalation of Adenovirus-driven Cre. The effects on lung cancer development were investigated using histopathology, metabolomics and flow cytometry.

Results and discussions We found that, while loss of Keap1 alone displayed no abnormalities in the lung, loss of Keap1 combined with loss of the tumour suppressor Pten, promoted malignant transformation. We further monitored tumour progression and immune infiltration in the lung, and metabolite profile changes in the serum of the Keap1f/f/Ptenf/f mouse model. Notably, a tumour-specific metabolite signature was identified in the plasma of Keap1f/f/Ptenf/f tumour-bearing mice, which indicated that tumourigenesis is associated with metabolic reprogramming. Furthermore, the immune milieu was dramatically changed by Keap1 and Pten deletion, and tumour regression was achieved utilising immune checkpoint inhibition.

Conclusion Our study highlights the ability to exploit both metabolic and immune characteristics in the detection and treatment of lung adenocarcinomas harbouring KEAP1/NRF2 pathway alterations.

Abstracts

PO-211 KRAS DRIVEN LUNG ADENOCARCINOMA DEPENDS ON ERBB SIGNALLING

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PO-212 CEP55 OVER-EXPRESSION CAUSES GENOMIC INSTABILITY TO INITIATE SPONTANEOUS TUMOUR FORMATION IN VIVO

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Introduction CEP55, discovered first by our laboratory, is a key regulator of cytokinesis, with perturbation of its levels causing cytokinesis failure and multi-nucleation. Since its discovery, CEP55 over-expression has been linked to increased aggressiveness of multiple tumour types. This overexpression has been associated with increased AKT activation, caused by the interaction of CEP55 with p110 and promoting stability of the catalytic subunit of PI3KCA. Further, it has been shown that wild-type p53 suppresses CEP55 expression by negatively regulating PLK1 creating a p53-PLK1-CEP55 axis to directly modulate CEP55 stability and cytokinesis completion. In addition, CEP55 has been shown to be part of the CIN70 gene signature that predicts chromosomal instability and poor prognosis of several tumours. However, despite these studies, it is currently unknown whether elevated Cep55 levels alone are sufficient to promote de novo tumorigenesis.

Material and methods We have generated the first novel transgenic mouse model with overexpression of Cep55 from the ubiquitously expressed Rosa26 locus.

Results and discussions We observed that Cep55 over-expression in vivo leads to a wide-spectrum of spontaneous tumour formation with a long latency period. In addition, we have generated a bi-transgenic mouse model (Cep55Stg/Tg Trp53+/-) to determine the contribution of p53 loss to Cep55-induced human KRAS mutated advance tumours (stage II and more advance AC) were enriched in the ERBB gene expression signatures compared to stage I tumours. Experimentally, we found that genetic deletion of EGFR in a GEMM of KRAS driven lung AC or in human xenografted A549 cells significantly reduced tumorigenesis, irrespective of the p53 status. Tumours lacking EGFR showed less cell proliferation and reduce activation of KRAS downstream effectors. Pharmacological inhibition of EGFR using erlotinib or gefitinib failed to inhibit tumorigenesis in KRAS driven lung AC experimental models. On the contrary, afatinib (an irreversible pan-ERBB inhibitor) was effective in human/murine cell lines, xenograft, PDXs and GEMMs models. Detailed analysis of this observation revealed that genetic deletion or pharmacology inhibition (erlotinib/gefitinib) of the EGFR in KRAS driven lung AC results in a tumour-escape mechanism relying in the activation of other ERBB receptor family members (namely ERBB2 and ERBB3) that can be blocked with the pan-ERBB inhibitor afatinib.

Conclusion In conclusion, our data shows that KRAS driven lung AC is depending on ERBB signalling. Importantly, we have unravelled a tumor-escape mechanism depending on the (re)activation of non-EGFR ERBB receptors that explains the poor results in previous clinical trials using erlotinib and gefitinib in patients suffering KRAS mutated lung AC. In agreement with this, pan-ERBB inhibition using afatinib effectively abrogates KRAS driven lung AC. Thus, afatinib or other pan-ERBB inhibitors should be a therapeutic option to treat patients suffering of KRAS driven lung AC.