INHIBITION OF THE MTORC1-PATHWAY CAN FEEDBACK-ACTIVATE H-RAS OR K-RAS

Introduction PI3K/mTORC1- and Ras/MAPK-signalling pathways are aberrantly regulated in most cancers. Specific resistance drugs targeting these pathways can emerge during tumour evolution. Preexisting, innate resistance mechanisms, such as stemming from feedback-loops, should ideally be known already during drug-target nomination. However, as the example of B-Raf-inhibitors that paradoxically activate MAPK-signalling has shown, feedback mechanisms may only become apparent at very late drug development stages.

Material and methods HEK cells expressing FRET-pairs of Ras proteins were used to study specific effects on Ras isoforms (nanoclustering). Breast cancer cells were grown in 2D for Western blotting of Ras and mTORC1-pathway proteins, or as spheres to analyse stemness traits.

Results and discussions Here, we describe two broad feedback loops from the mTOR-pathway back to the nanoscopic membrane signalling complexes (nanocluster) of H-ras and K-ras4B (hereafter K-ras). Increased nanoclustering typically correlates with increased Ras output. The first, upstream loop leads to an inadvertent rapalog induced promotion of stemness traits and tumorigenicity in Ras transformed cells. This is due to an induction of the H-ras nanocluster scaffold galectin-1, when FKB12 levels are low. Surprisingly we find that rapalogs do not only bind to but induce a loss of FKBP12 protein. Thus, rapalog treatment induces galectin-1, which stimulates H-ras signal output and stemness traits.

Secondly, modulation of the activity in the mTORC1 pathway downstream of the major lipid regulator SREBP1, oppositely regulates H-ras and K-ras nanoclustering. Thus, ablation of SREBP1 increases K-ras, but decreases H-ras nanoclustering and signal output. We show that altered levels of phosphatidic acid downstream of SREBP1 are sufficient for the opposite regulation of the two Ras isoforms.

Conclusion The described feedback loops may only become apparent in certain tumour settings. For example, tumour promotion during rapalog-treatment may only be relevant in H-ras mutant cancers, which make up a small portion of human cancers. In those cases, rapalog efficacy may be improved in combination with novel anti-galectin-1 drugs. Targeting the mTORC1 pathway downstream of SREBP1, may have opposite effects in H-ras and K-ras mutant cancers. Thus, care may have to be taken when targeting the mTORC1-pathway in a mutant Ras setting.

PO-094 PERSISTENT MYC ACTIVITY PREVENTS RESOLUTION OF LIVER INJURY

Introduction Despite individual tumours harbour a bewildering array of genetic alterations it is remarkable that tumours arising in the same tissue are very similar and different to tumours arising in other tissues. This suggests that the evolution of cancer is significantly restricted by its tissue of origin. Wound healing and tissue regeneration are characterised by profound changes, similar to events characterising the formation and development of many solid tumours. Given Myc’s role in proliferation, injury and cancers we have investigated its relationship with healing and tissue regeneration are characterised by profound changes, similar to events characterising the formation and development of many solid tumours. Given Myc’s role in proliferation, injury and cancers we have investigated its relationship with wound healing and, in particular, with the stromal changes that accompany tissue regeneration and cancers.

Material and methods In vivo studies were conducted with switchable GEMMs, where Myc activity can be regulated at will, directly and specifically in liver epithelial cells (RosamMycER;AlbuminCRE). Acute liver injury was induced by CCl₄. Experimental methodologies include: Histology, Cyto-kine array, Immune-cell isolation and RNA-seq.

Results and discussions In stark contrast to injured only animals, enforced maintenance of physiological Myc activity in the hepatocyte compartment completely blocked resolution of liver injury: tissue failed to normalise, necrotic debris persisted, liver damage markers remained elevated and hepatocytes remained in cycle. These ‘open wounds’ where characterised by dramatic influx of monocytes, exclusion of adaptive lymphoid cells, Stellate cells activation and impaired clearance of debris, closely phenocopying the regenerative phase of injury alone. Hence, the inability to down-regulate Myc after injury profoundly blocks resolution.