**OVERVIEW**

**KRAS** oncogenes have been implicated in about one fifth of all human cancers including lung and pancreatic adenocarcinomas, 2 of the tumour types with the worst prognosis. Unfortunately, identification of suitable therapies to treat these tumours remains elusive and patients are still treated with cytotoxic compounds approved over 2 decades ago. The recent discovery that these tumours display intra-tumour heterogeneity adds another layer of complexity that needs to be addressed. Hence, our laboratory has decided to search for novel therapeutic targets that may contribute to the early stages of lung tumour development, hoping that these targets will be present in all tumour cells – including cancer initiating cells and cancer stem cells – and not only in limited populations of evolving clones. In addition, we have continued our quest to validate known targets (mainly those of the MAPK and PI3K pathways) using genetically engineered mouse tumour models with the ultimate goal of establishing rational combination therapies that may provide significant therapeutic benefits in the clinic.

**Significance**

- We have shown that human lung tumours respond efficiently to combinations of DDR1 and NOTCH inhibitors in PDX models.
- We have provided a mechanistic explanation for the exclusive presence of K-RAS or EGFR mutations in human lung adenocarcinomas.
- We have demonstrated that the different incidence of H-RAS and K-RAS oncogenes in human tumours is due to the signalling intensity of their respective oncoproteins.
Identification of novel therapeutic targets for the treatment of K-Ras driven lung adenocarcinoma.

The recent discovery that lung tumours display significant levels of clonal heterogeneity (Govindan, Science, 2014) implies that effective therapies must target early oncogenic events/alterations present in all tumour cells and not only in clonal variants that appear during tumour development. To provide potential solutions to this key issue we decided to search for novel therapeutic targets present in the earliest stages of lung tumour development, expecting that such targets will be present in the entire tumour population including the putative cancer initiating/stem cells. Among the most highly expressed druggable genes we identified Ddr1, a locus that encodes a tyrosine protein kinase receptor. As reported early this year (Ambrogio et al., Nat. Med., 2016), genetic and pharmacological inhibition of Ddr1 prevented progression of K-Ras driven p53 wild type, but not p53 mutant tumours. Yet concomitant inhibition of Ddr1 and Notch, a downstream mediator of Ddr1 activity, led to a significant anti-tumour effect even in aggressive K-RasG12V, p53 mutant adenocarcinomas. More importantly, this treatment induced regression of K-Ras, p53 mutant patient-derived lung orthotopic xenografts (PDOX) with a therapeutic efficacy superior to standard chemotherapy. Identification of additional early oncogenic targets present in these early K-Ras mutant driven lung cells should expand the therapeutic opportunities to treat K-Ras mutant tumours in the clinic, thus by-passing the challenges derived from the development of intra-tumour heterogeneity.

Lack of selective advantage for lung cells expressing K-Ras and EGFR oncogenes.

Activating mutations in KRAS and EGFR, the 2 most frequent oncogenic drivers in human lung adenocarcinoma, occur in a mutually exclusive manner suggesting functional redundancy and implying lack of positive selection. By means of a mouse model engineered to induce expression of mutant EGFR in advanced tumours driven by a resident KnasG12V oncogene, we showed that, instead, their co-expression is detrimental for Kras and implying lack of positive selection. By means of a mouse xenograft model, the 2 most frequent KRAS K- and L858R in oncogenes are involved in different human tumour types. To determine whether the oncogenic versions of the H-Ras and K-Ras proteins are also bioequivalent, we knocked-in H-RasG12V oncogene sequences into the K-Ras locus. Germline expression of H-RasG12V or K-RasG12V from the K-Ras locus resulted in equal embryonic lethality. However, their expression in adult mice led to different tumour phenotypes. Whereas H-RasG12V elicited papillomas and haematopoietic tumours, K-RasG12V induced lung tumours and gastric lesions. The reason why H-RasG12V expression failed to cause lung tumours is due to the induction of a senescence-like state due to excessive MAP kinase signalling. Likewise, H-RasG12V but not K-RasG12V induced oncogene-induced senescence in mouse embryonic fibroblasts (MEFs). Label-free quantitative analysis revealed that minor differences in H-RasG12V expression levels led to drastically different biological outputs, suggesting that subtle differences in MAP kinase signalling influence the differential tumour spectra induced by RAS oncogenes.

Whereas the wild type H-Ras and K-Ras proteins are bioequivalent, their oncogenic isoforms H-RasG12V and K-RasG12D induce different tumour spectra.

We have provided genetic evidence demonstrating that the H-Ras and K-Ras proteins are fully bioequivalent in mice. Previous studies have shown that replacement of the K-Ras alleles by H-Ras coding sequences resulted in viable mice (Potenza et al., EMBO Rep. 2005). Yet, these mice displayed cardiovascular defects. Nor, have we shown that these defects were due to the presence of the 4 H-Ras expressing alleles in these mice. Ablation of the 2 endogenous H-Ras alleles, hence generating mice that only express the H-Ras protein from the 2 targeted K-Ras alleles, is absolutely normal. These results appear to be at variance with the well-established observation that H-Ras and K-Ras oncogenes are involved in different human tumour types. To determine whether the oncogenic versions of the H-Ras and K-Ras proteins are also bioequivalent, we knocked-in H-RasG12V oncogene sequences into the K-Ras locus. Germline expression of H-RasG12V or K-RasG12V from the K-Ras locus resulted in equal embryonic lethality. However, their expression in adult mice led to different tumour phenotypes. Whereas H-RasG12V elicited papillomas and haematopoietic tumours, K-RasG12V induced lung tumours and gastric lesions. The reason why H-RasG12V expression failed to cause lung tumours is due to the induction of a senescence-like state due to excessive MAP kinase signalling. Likewise, H-RasG12V but not K-RasG12V induced oncogene-induced senescence in mouse embryonic fibroblasts (MEFs). Label-free quantitative analysis revealed that minor differences in H-RasG12V expression levels led to drastically different biological outputs, suggesting that subtle differences in MAP kinase signalling influence the differential tumour spectra induced by RAS oncogenes.