Editorial

In vivo experimental models account for higher complexity than *in vitro* preclinical settings in cancer

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Introduction

Lung cancer is the leading cause of cancer death worldwide. There is no current treatment able to efficiently treat the disease as the lung cancer is often diagnosed at an advanced stage. Furthermore, lung cancer cells are often resistant or acquire resistance to the treatment. There is an unprecedent need to understand the mechanisms driving lung tumorigenesis, aggressiveness, metastasization, and resistance to treatments as they could provide new tools for detecting the disease at an earlier stage and for a more tailored and successful therapy. *KRAS* mutations are the most frequent oncogenic aberrations in lung cancer patients. However, even if this oncogenic driver and its biology have been characterized since long time, so far, no therapy targeting hyperactivated KRAS has been developed to efficiently treat the disease (1). The reason for this is that Ras controls multiple oncogenic signalling, as well as apoptotic/senescence signalling as a negative feedback mechanism. In this scenario, targeting only one of those signallings does not reveal much effectiveness in clinics. Thus, it is important to extend the knowledge of Ras biology and to identify new pathways mediated by Ras or intersecting with Ras signalling, to find possible synthetic lethal partners for a more effective RAS targeted therapy. Ras association domain family 1 isoform A (RASSF1A) is a tumor suppressor found to be epigenetically inactivated in many tumor types, including human lung cancers (2-5). It physically associates with oncogenic KRAS, and mediates apoptosis and senescence thereby providing a feedback mechanism that opposes excessive oncogenic Ras activity. RASSF1A oncosuppressive function is elicited through multiple mechanisms. On one hand it functions as a scaffold protein for KRAS in the direct activation of pro-apototic pathways, on the other hand it indirectly modulates KRAS mediated pro-mitogenic signalling through the modulation of Ras mitogenic effectors (PI3K/AKT, MAPK/ERK, RALGEF) (6). RASSF1A also links RAS to the Hippo pathway, while RASSF1A loss uncouples Ras from this oncosuppressive pathway (7-10). Finally, it has been shown that RASSF1A attenuates pro-inflammatory events (11), even if the role of RASSF1A in the control of Ras-driven inflammation is yet unexplored. Lung cancer patients with activated oncogenic Ras and reduced RASSF1A expression exhibit poor prognosis (12). However, *in vivo* evidence of the oncosuppressive role of RASSF1A in a mutant KRAS context in lung is still scarce.

RASSF1 inactivation *in vivo* increases KRAS driven tumorigenesis through unidentified pathways that are independent on MAPK, RAL and YAP activation

Shmidt *et al.* originally analysed *in vivo* the mechanisms through which RASSF1A acts as a tumor suppressor in KRAS driven tumors. They elegantly report that in RAS12D+ mice, RASSF1A haploinsufficiency (which

recapitulates its epigenetic silencing observed in human tumors) enhances the tumorigenicity of KRAS (measured as an increased number of tumor masses, with no apparent increase in tumor average size (*Figure 1A*, left and right panels) as well as the KRAS-related inflammation. Conversely, in tumoral and in non-tumoral tissues in which RAS is not hyperactivated, loss of RASSF1A only upregulates the RAS mediated mitogenic pathways (to the same fashion observed in mutant KRAS background) without any detectable effects on tumorigenesis (*Figure 1B*, left and right panels). The authors do not clarify what pathways are differentially affected in the KRAS wt *vs.* mutated background upon RASSF1A inactivation. Thus, the reported evidences do not provide any specific mechanism to explain the role of KRAS/RASSF1A axis in lung cancer.

In particular, the authors do not describe any mechanism that can be responsible of lung tumorigenesis in KRAS mutated but not in KRAS wt background upon RASSF1A inactivation.

In detail, the authors report that the loss of RASSF1A determines:

- ❖ an increased expression of the oncogenic YAP in KRAS mutant tumors. In the same experimental setting expressing RASSF1A, YAP is maintained at lower levels. This is because RASSF1A mediates RAS-induced activation of the Hippo pathway as a feedback mechanism;
- ❖ an increase of the mitogenic AKT phosphorylation and MAPK activation;
- ❖ an increased expression of Ras related RAL oncoproteins;
- ❖ an augmented RAS-mediated inflammation as increased CD11 infiltration and IL-6 expression (this latter effect was observed both in murine system *in vivo* and in human cell lines *in vitro*).

However, the effects on YAP, AKT and RAL signalling occur both in KRAS wt and in mutated contexts, suggesting that these mitogenic pathways, at least in this experimental system, are not responsible for driving the increased tumorigenesis observed in the KRAS mutant background compared to its wt counterpart. This strongly suggests that KRAS hyperactivation and concomitant loss of RASSF1A trigger other pathways that need to be investigated in order to explain the different behaviour of KRAS mutated *vs.* KRAS wt lung tumor upon RASSF1A inactivation (*Figure 1*). Moreover, this provides further evidence that tumor biology *in vivo* is much more complex and cannot always be explained through the simple dissection of molecular mechanisms *in vitro*. Indeed, such complexity might have important implications in clinics. Based on this, the authors suggest that it is necessary to take into account the KRAS activation status of the lung tumor in order to define the best therapeutic approach. This reinforces the general emerging concept that precise information about the genetic and epigenetic landscape of each tumor is required to better predict the response to different targeted therapies and to design the best therapeutic options for each patient. Moreover, this work opens the road for new studies aimed at investigating what other pathways can be differentially affected in the KRAS wt or mutated background upon RASSF1A inactivation. This might lead to the identification of those signalling pathways responsible for a more *in vivo* aggressive phenotype as such due to RASSF1A inactivation in a KRAS mutated background.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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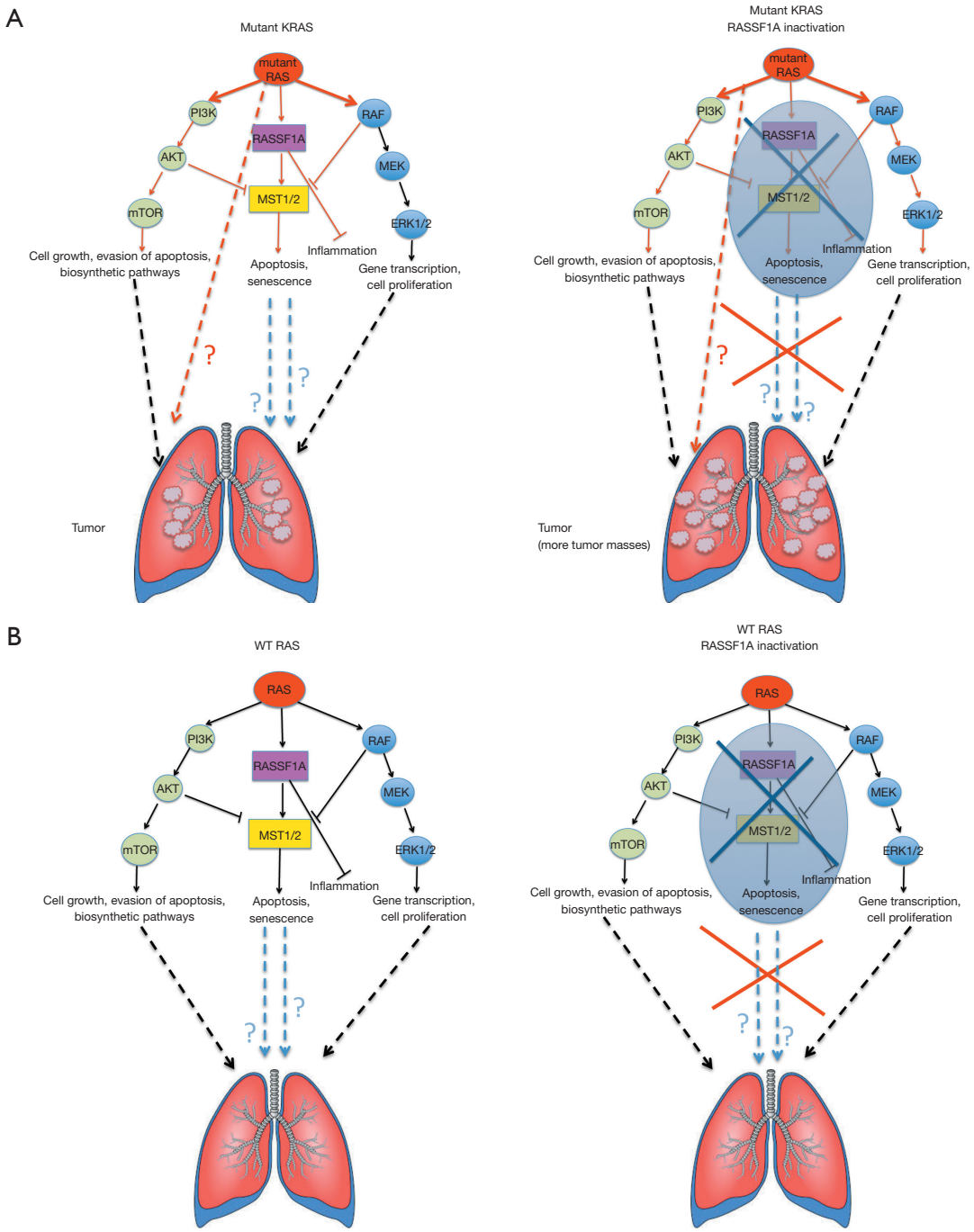

Figure 1 Schematic representation of pro-mitogenic and pro-apoptotic signaling orchestrated by Ras and RASSF1A. Well characterized signallings are described in the figure, while dashed arrows with question marks indicate yet uncharacterized mechanisms mediated by mutant KRAS (arrows in red) of by RASSF1A (arrows in blue). Based on findings by Shmidt *et al.*, RASSF1A inactivation in a KRAS wt background is not sufficient to drive lung cancer (B, left and right panels). KRAS hyperactivation drives lung cancer development through the hyperactivation of pro-mitogenic and/or antiapoptotic signallings, some of which are still poorly characterized (dashed arrow in red, *Figure 1A*, left and right panels). In KRAS mutant background, RASSF1A inactivation further increases the number of tumor masses driven by KRAS mutation because it inactivates oncosuppressive/proapoptotic mechanisms that are still unexplored *in vivo* (dashed arrows in blue; A, right panel).

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