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COMMENTARY



# The mystery of oncogenic *KRAS*: Lessons from studying its wild-type counter part

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**ABSTRACT**

Using conditional knock-in mouse models, we and others have shown that despite the very high sequence identity between Nras and Kras proteins, oncogenic Kras displays a much stronger leukemogenic activity than oncogenic Nras in vivo. In this manuscript, we will summarize our recent work of characterizing wild-type Kras function in adult hematopoiesis and in oncogenic Kras-induced leukemogenesis. We attribute the strong leukemogenic activity of oncogenic Kras to 2 unique aspects of Kras signaling. First, Kras is required in mediating cell type- and cytokine-specific ERK1/2 signaling. Second, oncogenic Kras, but not oncogenic Nras, induces hyperactivation of wild-type Ras, which significantly enhances Ras signaling in vivo. We will also discuss a possible mechanism that mediates oncogenic Kras-evoked hyperactivation of wild-type Ras and a potential approach to down-regulate oncogenic Kras signaling.

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In mammals, there are 3 *Ras* genes (*Hras, Nras*, and *Kras*) encoding 4 homologous 21kD proteins: Hras, Nras, Kras4A and Kras4B.[1] The first 85 amino-terminal residues are identical and the middle 78 amino acids share an 85% – 90% identity among all Ras isoforms, while the last 25 amino acids at the carboxyl-terminus are highly variable.[2] In haematopoietic neoplasms, oncogenic mutations in the *NRAS* and *KRAS* genes are common, but rare in the *HRAS* gene.[2] Despite the high similarity in protein sequences and largely overlapping expression patterns, accumulating evidence suggest that $Kras^{G12D/+}$ expressed from its endogenous locus demonstrates much stronger leukemogenic activity than oncogenic *Nras* alleles (including $Nras^{G12D}$ alleles) (Fig. 1). Using conditional knock-in mouse models, we and others characterized multiple oncogenic *Ras* alleles in a pure C57BL/6 background, including the weak $Nras^{G12D/+}$, the intermediate $Nras^{Q61R/+}$ and $Nras^{G12D/G12D}$, and the strong $Kras^{G12D/+}$.[3-8] All of these models involve a conditional knock-in oncogenic *Ras* allele and the interferon-inducible Mx1-Cre. Upon polyinosinic-polycytidylic acid (pI-pC) injections to induce the expression of Cre and subsequently the oncogenes, $Kras^{G12D/+}$ mice die rapidly, while $Nras^{G12D/G12D}$ and $Nras^{G12D/+}$ mice show incrementally prolonged survival (Fig. 1A). To eliminate the impact of acute interferon signaling on animal survival, we also took advantage of the leaky expression of Mx1-Cre over the time. Again, the non-pI-pC treated $Kras^{G12D/+}$ mice display a significantly shorter survival than the corresponding $Nras^{G12D/G12D}$ mice (Fig. 1B). These results clearly demonstrate that oncogenic *Kras* is a much more potent oncogene than oncogenic *Nras* in leukemogenesis.

To decipher the unique function of oncogenic Kras signaling in leukemogenesis, we first investigated how loss of wild-type (WT) Kras impacts on adult hematopoiesis.[9] We found that loss of *Kras* leads to greatly reduced TPO signaling in haematopoietic stem cells (HSCs), while SCF-evoked ERK1/2 activation is not affected. The compromised TPO signaling is associated with reduced long term- and intermediate-term HSC compartments and their reduced self-renewal capability (Fig. 2). Although GM-CSF-evoked ERK1/2 activation is only moderately decreased in $Kras^{-/-}$ myeloid progenitors, it is blunted in neutrophils and neutrophil survival is significantly reduced in vitro. Similarly, the ERK1/2 pathway is down-regulated in $Kras^{-/-}$ B cells, which is associated with B cell differentiation defects at both pre-B cell and mature B cell stages.[10] These results reveal a surprising, indispensible role of Kras in mediating cell type- and cykokine-specific ERK1/2 activation, especially in differentiated cells (e.g. neutrophils). This conclusion is further supported by the finding that in $Kras^{-/-}$; $Nras^{Q61R/+}$

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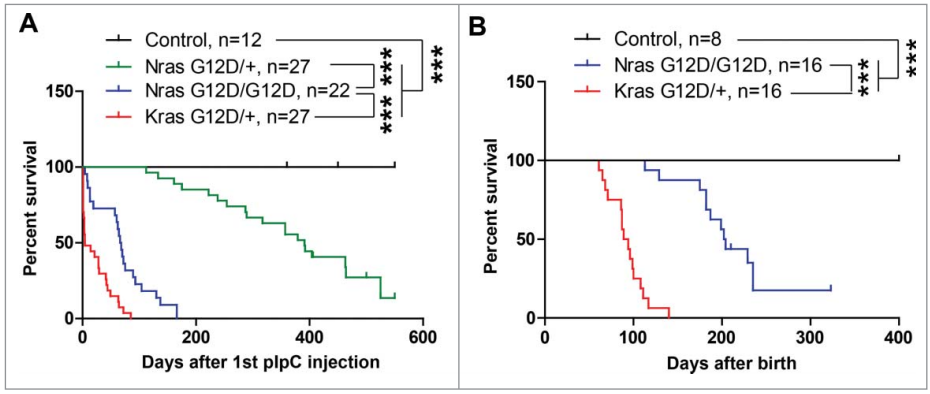


**Figure 1.** Oncogenic *Kras* displays more potent leukemogenic activity than oncogenic *Nras*. (A) Six-seven weeks old control, $Kras^{G12D/+}$, $Nras^{G12D/+}$, and $Nras^{G12D/G12D}$ mice were injected with pI-pC twice every other day. Kaplan-Meier survival curves were plotted against days after 1st pI-pC injection. (B) Kaplan-Meier survival curves of non-pI-pC treated control, $Kras^{G12D/+}$, and $Nras^{G12D/G12D}$ mice. P values were determined using the Log-rank test. *** $P < 0.001$.

neutrophils, GM-CSF-evoked ERK1/2 activation remains blunted (our unpublished result).

Interestingly, at 9–12 months old, a fraction of *Kras* conditional knockout mice develop profound haematopoietic defects (e.g., splenomegaly and an expanded neutrophil compartment), suggesting that they are highly prone to myeloid diseases. Indeed, we recently found that loss of WT *Kras* promotes oncogenic *Kras*-induced myeloproliferative neoplasm in a cell autonomous manner.[11] The most striking result from this study is that oncogenic Kras but not oncogenic Nras leads to cross-activation of WT Ras in total bone marrow cells, while loss of WT *Kras* further promotes the activation of all Ras isoforms. Previous crystallographic analyses of Ras bound to the catalytic module of Sos indicate that Ras-GTP binds to the catalytic domain of Sos at a site distal to the active site of Sos and stabilizes this active site allosterically, significantly increasing the rate of Sos-stimulated GDP release from Ras.[12] Subsequently, Bar-Sagi's group reported that cross-activation of WT Ras by oncogenic Ras is mediated by Sos1 and is essential for oncogenic *Ras*-induced tumorigenesis in cancer cell lines[13] (Fig. 3). Our recent in vivo work is also in agreement with this conclusion (our unpublished results). Of note, the earlier studies imply that cross-activation of WT Ras is applicable to all Ras isoforms, while our in vivo study demonstrates that this phenomenon is specific to oncogenic Kras in total bone marrow cells. We speculate that this specificity might be related to the differential membrane trafficking and/or localization of different Ras isoforms, which is mediated by their highly variable C-termini.[14-19]

To our surprise, loss of WT *Kras* not only further promoted activation of WT Hras and Nras but also significantly enhanced oncogenic Kras-GTP levels. It is known that $Ras^{G12D}$ mutation affects the intrinsic GTPase activity of Ras proteins by preventing proper position of the Ras-GAP (GTPase activating protein) arginine finger within its catalytic site.[20-22] Conceivably, although majority of $Kras^{G12D}$ protein binds to GTP, a fraction of $Kras^{G12D}$ protein binds to GDP and is inactive. One possibility is that all GDP-bound forms of Ras, including WT Ras and a small proportion of $Kras^{G12D}$, compete for the binding to the activate site of Sos catalytic domain to release GDP. Without WT Kras,

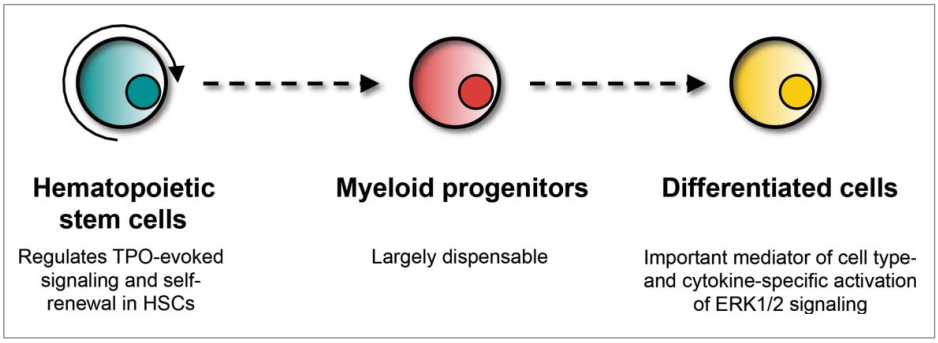


**Figure 2.** Summary of wild-type Kras function in adult hematopoiesis.

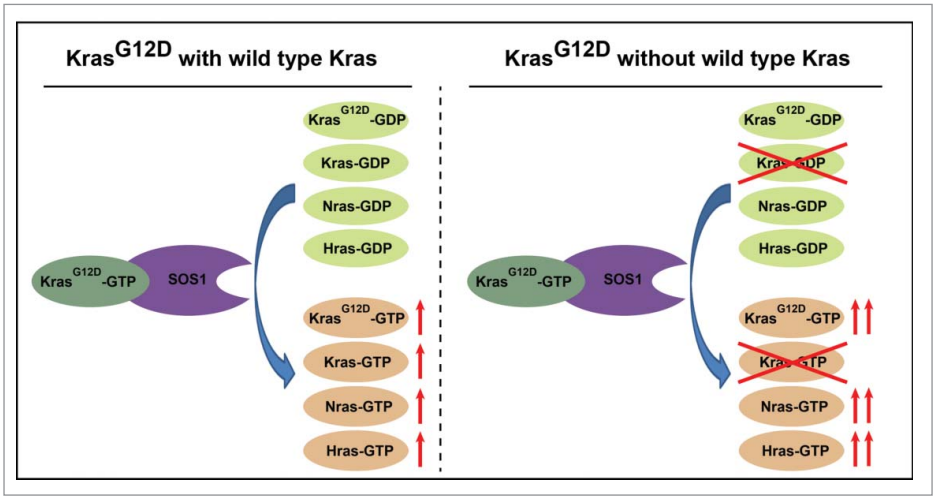


**Figure 3.** Schematic illustration of a potential mechanism how loss of WT *Kras* promotes activation of all Ras isoforms.

inactive WT Hras and Nras as well as inactive $Kras^{G12D}$ have a higher chance to be activated by Sos (Fig. 3). Alternatively, it could be that expression level of oncogenic Kras from a single copy of the oncogene is upregulated to a level comparable to that from 2 copies of the oncogene, as suggested by our quantitative Western blot analysis.[11] The mechanism underlying this upregulation is unknown, but perhaps mediated through an epigenetic regulation. We would like to point out that these 2 possibilities are not mutually exclusive. In fact, it is highly likely that both of them contribute to the higher Kras-GTP level we observed.

Our work demonstrates that in bone marrow cells, expression of oncogenic Kras is equivalent to the activation of all Ras isoforms, including WT Nras and Hras, which could significantly contribute to the potent leukemogenic activity of oncogenic Kras. The question is "Can we target the interaction between oncogenic Kras and Sos1 to ameliorate oncogenic Kras-induced leukemias?" The answer to this question might be complex. In one hand, inhibition of this interaction could block activation of WT Ras and alleviate at least some of the leukemia phenotypes. On the other hand, due to the unique function of oncogenic Kras signaling in certain cell types as described above, inhibition of the interaction between oncogenic Kras and Sos1 would have none or minimal effects in these cells. Therefore, our data suggest that targeting this interaction should be combined with other therapies, such as inhibitors targeting Ras down stream MEK1/2, to achieve more potent anti-leukemia effects.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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