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AUTHOR'S VIEW

NRAS-driven melanoma: A RAF can hide another

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ABSTRACT
Using mouse genetics, we recently showed that BRAF has a critical role in initiation of NRAS-driven melanoma that cannot be compensated by CRAF. In contrast, RAF proteins display compensatory functions in fully established tumors and ARAF can sustain proliferation in the absence of BRAF and CRAF, highlighting an addiction to RAF signaling in NRAS-driven melanoma.

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Melanoma arises from malignant transformation of melanocytes, which are specialized pigment-producing cells derived from the neural crest. The incidence of cutaneous melanoma, the deadliest of skin cancers, is continuously increasing especially in western countries. It is an heterogeneous disease that displays one of the highest mutation load of any cancer.[1] However, constitutive activation of the RAS/RAF/MEK/ERK pathway represents a hallmark of cutaneous melanoma, with recurrent and mutually exclusive driver mutations in either NRAS or its downstream effector BRAF. BRAF is a member of the RAF family of MEK activators, which also comprises ARAF and CRAF.[2] Research on RAF proteins in the field of melanoma has boomed since the discovery of BRAF mutations and the recent development of highly specific inhibitors, such as Vemurafenib and Dabrafenib.[3] However, these inhibitors cannot be used in non BRAF-mutated melanoma patients, including those carrying an NRAS mutation, and even cause toxicities due to paradoxical MAPK/ERK signaling pathway activation by BRAF-CRAF heterodimers.[3] Furthermore, in most RAS-mutated cancers where the contribution of RAF kinases has been examined so far, either in human cell lines or in mouse models, CRAF, but not BRAF, was shown to be essential for MAPK/ERK activation.[4,5,6] Regarding melanoma, *in vitro* studies suggested that CRAF could be also critical for NRAS-mutated tumor growth.[7] However, no CRAF driver mutation has been found so far in cutaneous melanoma, whereas NRAS and BRAF are mutated in more than 15% and 50% of cases, respectively. Finally, we reported that BRAF and CRAF act in a redundant manner to ensure normal melanocyte stem cell self-maintenance.[8]

Taken together, these observations raised an intriguing paradox whereby BRAF could be dispensable for NRAS-induced melanoma, while it plays a key role in normal melanocytes and in BRAF-induced melanoma. Therefore, in a recent study,[9] we decided to reevaluate the respective contributions of RAF kinases in NRAS-induced melanoma using mouse models allowing conditional ablation of both BRAF and CRAF genes in the melanocyte lineage and recapitulating the different phases of melanoma progression. These complex models involving up to 6 loci and 9 different alleles led us to demonstrate that the role of RAF kinases in NRAS-induced melanoma is much more complex than described previously. In these models, expression of $NRAS^{Q61K}$ is driven in the mouse melanocyte lineage through the tyrosinase promoter and rapidly induces early benign melanocytic lesions displaying all the characteristics of nevi.[10] On an $INK4a^{+/-}$ background, these lesions can eventually bypass NRAS-induced senescence to evolve toward malignant tumors. The $NRAS^{Q61K}$ and $INK4a^{+/-}$ mutations have been combined with BRAF and CRAF conditional knockouts and the contribution of both genes in tumor progression, from the onset of benign tumors to malignant tumor maintenance, was evaluated by their single or compound ablation upon tyrosinase promoter-driven Cre or Cre^{ERT2} expression.[9]

Our results showed that BRAF, but not CRAF, was critical for the formation of early skin melanocytic lesions, including nevi-like benign tumors, revealing a critical role for BRAF in NRAS-driven melanoma initiation that cannot be compensated by CRAF (Fig. 1a). In contrast, BRAF and CRAF display compensatory functions in fully established tumors, since only concomitant ablation of both BRAF and CRAF resulted in a complete blockage of tumor growth, a cooperative effect that was also observed in NRAS-mutated human melanoma cell lines (Fig. 1b,c). At the molecular level, we show that during early lesional stages, only BRAF can efficiently bind NRAS to ensure the required level of ERK activation, whereas both BRAF and CRAF form complexes with NRAS in fully established melanomas where the ablation of CRAF can be compensated by an increase in

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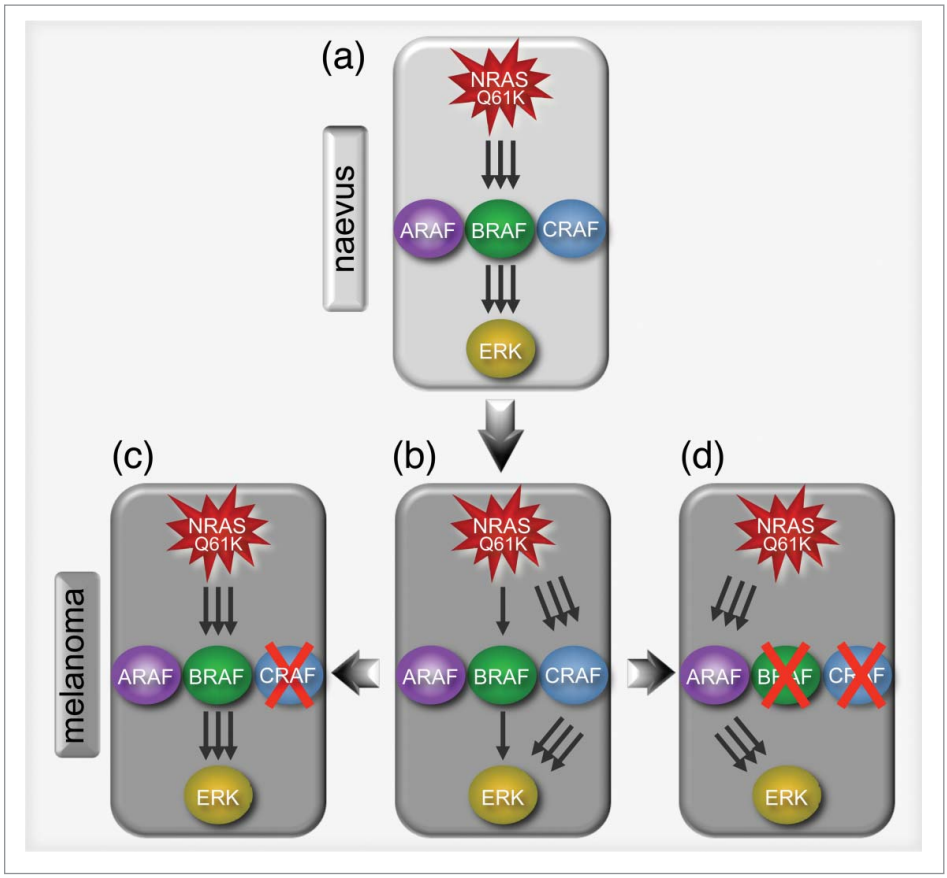

Figure 1. specific and compensatory functions of RAF proteins in NRAS-driven melanomas. (a) BRAF is required for tumor initiation: nevi cannot develop in the absence of BRAF in mice expressing $NRAS^{Q61K}$ in the melanocytic lineage. (b) in fully established melanoma, NRAS uses preferentially CRAF to activate the ERK pathway. (c) ablation of CRAF in NRAS-induced melanoma does not block tumor growth. An increase in the kinase activity of BRAF is sufficient to compensate for CRAF absence. (d) ablation of both CRAF and BRAF leads to the emergence of resistant clones, which proliferation is dependent on an ARAF-mediated ERK pathway activation.

BRAF kinase activity (Fig. 1c). Importantly, we further show that this capacity of BRAF to overcome the absence of CRAF is also observed in NRAS-mutated human melanoma cell lines. These results show that besides CRAF, BRAF is also important for sustaining established NRAS-driven melanoma growth, demonstrating compensatory functions for both kinases during later stages of tumor progression.

Interestingly, we further show that ablation of both BRAF and CRAF in NRAS-mutated melanoma cells, results, after a latency period, in the emergence of resistant clones, which proliferation is dependent on an ARAF-mediated MAPK/ERK pathway activation, providing the first evidence for a role of the third member of RAF family in melanoma (Fig. 1d). As mentioned above, RAF inhibitors such as Vemurafenib and Dabrafenib are not used to treat non BRAF-mutated melanoma patients because they induce paradoxical ERK activation, a process thought to be mediated by BRAF-CRAF dimerization. However, using double BRAF- and CRAF-deficient resistant cultures, we demonstrate that Vemurafenib is capable to activate both ARAF and ERK, thereby demonstrating that ARAF homodimers are sufficient to sustain ERK paradoxical activation in the complete absence of other RAF proteins.

In conclusion, our study is the first one to investigate the specific role of RAF proteins at different phases of tumor progression in a mouse model. Our findings disclose specific and complementary functions for RAF kinases in NRAS-induced mouse melanoma, which have not been observed in other RAS-mutated cancers such as lung cancer for example.[4,5] Taken together, they show that BRAF plays a key role in NRAS-driven melanomas and that these tumors are addicted to RAF signaling, an issue that will need to be taken into account for the development of future treatments based on targeted RAF inhibition, which are likely to generate potential resistance mechanisms.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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