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Shifting ecologies of malignant and nonmalignant cells following BRAF inhibition

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In this issue, Yaktapour et al. describe a patient with metastatic melanoma harboring the BRAF^{V600E} mutation who, upon treatment with the BRAF inhibitor vemurafenib, developed a sustained lymphocytosis that was ultimately diagnosed as del13q14 chronic lymphocytic leukemia (CLL) without mutations in *IGHV*. Notably, Yaktapour and colleagues demonstrate that SYK-mediated B cell receptor (BCR) signaling in CLL in the presence of drug-bound BRAF is a putative driver of the paradoxical MEK/ERK activation that has been occasionally observed in response to BRAF inhibition (1).

Current therapy

With the ever-expanding understanding of the genetic underpinnings of malignancies afforded by large-scale genome-wide sequencing studies and the concurrent expanding armamentarium of effective agents for targeted disease treatment, we have now definitively entered the age of precision medicine. Already, these advanc-

es have led to dramatic improvements in the clinical care of melanoma patients. For example, the selective BRAF mutation-specific inhibitor vemurafenib, which demonstrated unprecedented activity as a single agent in early phase trials, was approved by the FDA in 2011 and is now considered first-line therapy for patients with metastatic BRAF mutation-positive disease (2). Vemurafenib treatment has been impactful, as 50% of melanomas harbor a mutation that alters amino acid V600 of BRAF. The essential MAPK pathway, of which BRAF is a key component, normally functions to transduce growth signals from cell-surface receptors to the transcriptional machinery in the nucleus, promoting proliferation and survival. Activation of this pathway is mediated through the RAS GTPases, the serine/threonine kinase RAF, MEK, and the extracellular signal receptor kinase ERK. The canonical V600 mutation (V600E) in BRAF leads to a greater than 500-fold increase in BRAF catalytic activity and a subsequent con-

stitutive activation of MAPK pathway signaling; therefore, inhibition of BRAF^{V600E} with vemurafenib potentially eliminates this strong survival signal (Figure 1A).

While combination chemotherapy has been the mainstay for treatment of the B cell malignancy CLL for decades, the clinical efficacy of the recently FDA-approved single agent ibrutinib, which selectively and irreversibly inhibits Bruton's tyrosine kinase (BTK), an essential component of the BCR-signaling pathway, has marked the beginning of a new era in CLL treatment (3). In a recent trial of patients with relapsed, refractory CLL, ibrutinib induced an overall response rate of 71% and an estimated progression-free survival rate of 75% after 26 months of therapy (4). Likewise, other BCR signal-abrogating agents, such as inhibitors of the BCR-associated kinases SYK (5) and PI3K δ (6), have shown promising clinical anti-CLL activity. The dramatic impact of these agents in CLL underscores the key role of BCR signaling in driving the growth and proliferation of this common adult B cell leukemia.

Research advance

The same large-scale sequencing approaches that have revealed the somatic mutation landscape of cancers have also shown us that each individual tumor is composed of cellular subpopulations (7) defined by distinct genetic (8) and epigenetic differences (9), which together dictate the fitness of a subclone. The individual evolutionary trajectories of each tumor are affected by the traits of individual subpopulations, and exposure to strong selective pressure, such as persistent and potent BRAF inhibition, can provide a growth advantage to rare inhibition-resistant cells and the impetus for further evolution (10). Thus, the striking response of metastatic melanoma to vemurafenib (Figure 1B) results predominantly from the clonal status of BRAF mutations in melanoma, which are present in all or the vast majority of the cancer cell population. In contrast, BRAF resistance, which

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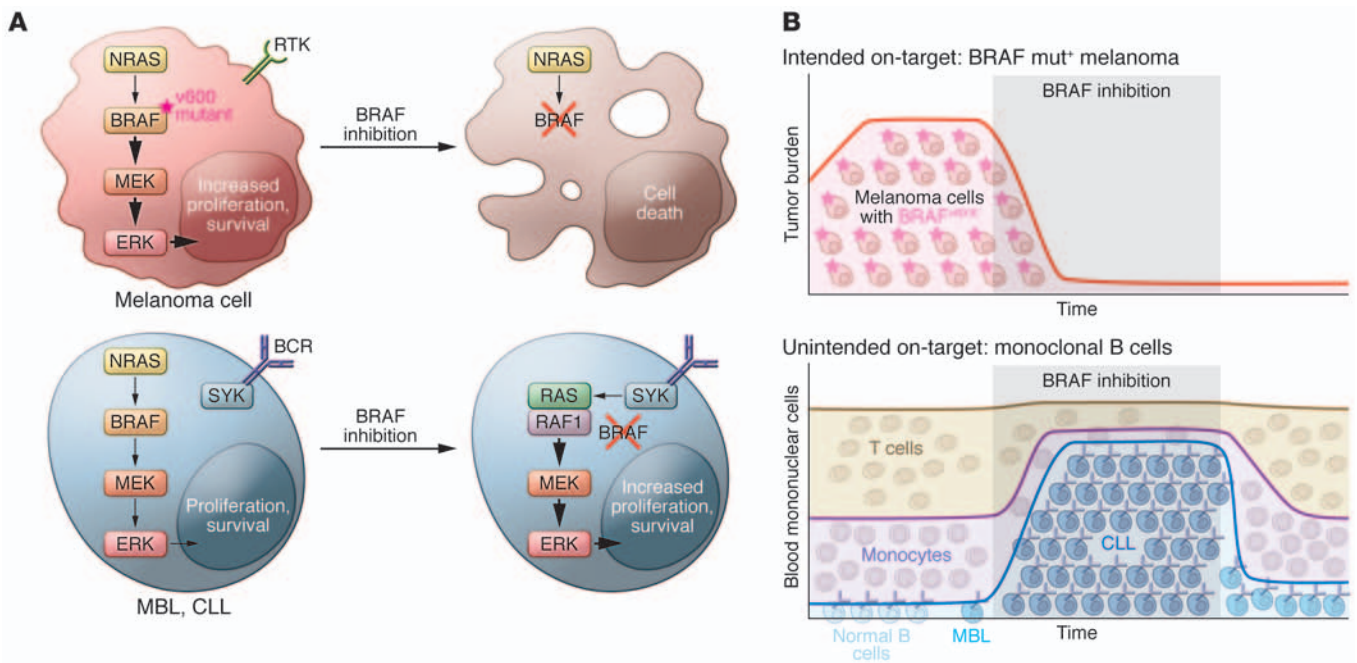


Figure 1. Shifting ecologies with vemurafenib exposure. (A) The mechanism of proliferation and survival through the MAPK signaling pathway in melanoma and CLL/MBL. In mutated BRAF⁺ melanoma, this pathway is constitutively activated through the effects of the V600E mutation, which is strongly activating. Exposure to BRAF-inhibiting agents, such as vemurafenib, disables this pathway, leading to cell death. CLL and MBL have autonomous BCR signaling. In the setting of BRAF inhibition, drug-bound BRAF and BCR/SYK-activated RAS cooperatively induce paradoxical ERK activation in CLL cells, leading to proliferation and survival. (B) Schematic representation of the temporal association between the development of B cell lymphocytosis/CLL with vemurafenib exposure and its regression with removal of the pharmacologic agent.

develops in many vemurafenib-treated patients, can be attributed at least in part to the existence of resistant subclones.

Although vemurafenib selectively inhibits malignant cells that express mutant forms of BRAF, all other cells in the recipient are also systemically exposed to this agent. Thus, even as vemurafenib eradicates the predominant BRAF mutation-positive population, the cellular circuitry of apparent nonmalignant populations may be affected as an unintended “on-target” effect. Previous reports have described the development of chronic myelomonocytic leukemia and squamous cell lesions in vemurafenib-treated BRAF^{V600E} melanoma patients who harbored RAS-activating mutations within cells of the myeloid and epithelial lineage, respectively, with paradoxical promotion of ERK activation within these cell populations and their subsequent expansion (11, 12). In this issue, Yaktapour and colleagues now describe a patient with BRAF^{V600E}-driven melanoma with a previously unapparent and clinically insignificant population of monoclonal B cells (Figure 1B). Vemurafenib treatment in this patient resulted in subsequent expansion of this

B cell population and frank CLL in the absence of activating RAS mutations. Evaluation of patient CLL cells revealed activation of ERK and enhanced proliferation in response to BRAF inhibition. BRAF inhibition-dependent CLL expansion required BCR activation, revealing a previously unappreciated crosstalk between the BCR- and MAPK-signaling circuits. Moreover, evaluation of CLL samples from additional patients confirmed a BCR-dependent induction of MAPK signaling in response to BRAF inhibition. In CLL, but not other B cell malignancies, the BCR was recently shown to signal autonomously (13). The results from Yaktapour and colleagues implicate the BCR proximal kinase SYK as the culprit of this unexpected crosstalk, as it couples the BCR and RAS pathways. Importantly, SYK inhibition reversed ERK hyperactivation and CLL proliferation (Figure 1A) and increased survival in a murine model (1).

Increasingly, premalignant states are recognized to exist for multiple blood malignancies, with incidence increasing with age. For example, monoclonal gammopathy of undetermined significance (MGUS) has been described as a precur-

sor lesion for multiple myeloma, and myelodysplastic syndrome is considered a precursor for acute myeloid malignancies. Likewise, individuals without clinical evidence of CLL can nonetheless harbor a persisting clonal population of B cells with surface markers and a subset of genetic features (i.e., del13q14) that are identical to CLL but clinically present in numbers lower than 5×10^9 cells/l (5000/ μ l). This diagnostic entity has been hotly debated in the CLL community, as there is a spectrum of clone sizes of the expanded B cell population that ranges from below 0.1×10^9 cells/l, which can be found in more than 10% to 20% of the population over 40 years of age, to between 0.5 and 5×10^9 cells/l, which is clinically evident as monoclonal B cell lymphocytosis (MBL) and represented in 0.2% of the population over 40 years of age (14). Whereas the former level of expanded B cells is clinically insignificant, individuals with B cells that have expanded into the latter range have an increased relative risk of bacterial infections, with 1% to 5% of individuals within this range progressing to CLL. The exact events propelling the MBL-to-CLL transition have not been well character-

ized. The effects of vemurafenib-induced BRAF inhibition described by Yaktapour et al. certainly serve to reinforce the idea that the delicately balanced genetic programs that maintain the stability of the MBL clone size can be toppled by the strong perturbation of pharmacologic intervention.

Recommendations

The case reported by Yaktapour et al. well illustrates the potency as well as the challenges of targeted therapy. Even as this approach can effectively inhibit cancer pathways, it can also affect normal cell circuitry in a fashion that promotes cancer in other tissues. There are many take-home points from this case. First, continued surveillance — not only of the tumor population but of other cellular compartments as well — in the setting of targeted therapy treatment is important. With the anticipated increase of vemurafenib use in adult populations, in which the presence of nonclinically evident clonal B cells is not uncommon, it is important to be aware of the possibility of stimulating the progression of MBL to frank CLL. Thus, any change in hematologic parameters either in the blood or soft tissue, such as lymph nodes, should be investigated promptly and the drug withheld until the cause of leukocytosis is clarified. Second, the likelihood of treatment dilemmas due to the appearance of a secondary cancer will necessitate the cessation of targeted inhibitor treatment, even in the presence of good response in the inciting malignancy. Fortunately, Yaktapour and colleagues have provided a potential and ready solution

in the circumstance of secondary CLL — clinically available SYK inhibitors to shut down the hyperactivated ERK pathway. Finally, there should be strong consideration of alternate therapeutic approaches. Clonal evolution, which is driven by the vast genetic heterogeneity within each patient's cancer, is a key feature of cancer; therefore, potent single targeted agents, such as vemurafenib, would be anticipated to provide only short-duration cancer control, as has been borne out by clinical studies. In the long run, combinatorial cancer treatments, including use of the growing arsenal of immune-based therapies that have the ability to eradicate malignant cells in a multipronged and exquisitely tumor-specific manner (15), are likely to be more efficacious.

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