Taming the Wild-Types: Targeting PAK1 in Melanomas That Lack BRAF Mutations

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Remarkable progress has been made recently in identifying the molecular drivers of melanomagenesis. High-throughput genomic screening has revealed melanoma to be a diverse collection of tumors initiated by distinct oncogenes (1). About half of all cutaneous melanomas have activating mutations in the serine/threonine kinase BRAF that drives tumor initiation and progression through the mitogen-activated protein kinase (MAPK) pathway. The next most prevalent melanoma oncogene is the GTPase NRAS, which is found in 15%–20% of cases (2). Other histological subtypes of melanoma, such as acral lentigious lesions arising subungually or on the palms or soles of the feet, or mucosal melanomas, tend not to harbor BRAF mutations and are sometimes dependent upon the genetic amplification of and/or activating mutations in the receptor tyrosine kinase c-KIT. Ocular melanomas lack oncogenic BRAF and are associated with deleterious mutations in the G-proteins GNAQ and GNA11 (3). Although driving oncogenic events have been identified for approximately 70% of all cutaneous melanomas, there remains a group of approximately 30% for which the initiating event has not been determined.

In this issue of the Journal, Ong and colleagues identify p21-activating kinase 1 (PAK1) as a potential driver of and therapeutic target in a subset of BRAF wild-type (WT) melanomas (4). Through an initial genetic and immunohistochemical analysis, PAK1 was observed to be either amplified or overexpressed in 9% and 26% of melanomas. Segregation of the specimens on the basis of mutational status revealed those with PAK1 amplification to be BRAF WT, with no BRAF mutant tumor samples exhibiting copy number gain (4). When the authors considered mutational status and PAK1 protein expression, the picture was more complex. Although good correlation was seen between PAK1 expression and BRAF WT status, this was not absolute and some BRAF mutant melanomas (4/40) showed elevation of PAK1 (4). Further analysis of the link between genotype and PAK1 expression showed a non–statistically significant negative trend with NRAS mutation. Intriguingly, a small cohort of melanomas was identified that harbored both NRAS and BRAF mutations and lacked PAK1 expression (4).

PAK1 was first identified as a downstream effector of the small GTPases Rac1 and CDC42 (5). It regulates the actin cytoskeleton, where it helps coordinate the many signals required to control cell shape, adhesion dynamics, and migration (5). PAK1 is an important mediator of cell survival through the phosphorylation (and inactivation of) the proapoptotic protein BAD (6, 7). A role for PAK1 in cancer initiation and progression has also been demonstrated, with studies showing its overexpression to promote the anchorage-independent growth of, and genetic abnormalities in, immortalized breast epithelial cells (8). Recent studies have also shown PAK1 to be amplified and overexpressed in subtypes of breast and lung cancers, with further work showing these tumors to be dependent upon PAK1 signaling for their survival (9). PAK1 is widely up-regulated in a number of other human malignancies, including hormone-dependent tumors such as prostate cancer, squamous cancer of the lung and skin, and melanoma (10). In uveal melanoma cell lines (which typically lack BRAF mutations), elevated PAK1 expression correlates with invasive potential, an effect that is reversed upon its knockdown by small interfering RNA (siRNA) (11).

Many melanomas are “addicted” to signals through the MAPK pathway for their initiation, growth, and survival (12). The mechanism by which the MAPK pathway is activated in melanoma is dependent upon the driving oncogene. In the majority of melanomas, MAPK signaling is activated directly via oncogenic BRAF, which when mutated, directly phosphorylates MEK (12). In melanomas with NRAS mutations, MAPK activity proceeds via CRAF and is dependent upon the simultaneous deregulation of protein kinase A signaling (which would otherwise phosphorylate and inactivate CRAF) (2, 13). In contrast, PAK1 activates MAPK signaling through the phosphorylation of both CRAF at Ser338 and MEK1 at Ser298 (9). Consistent with these findings, Ong and colleagues observed that siRNA knockdown of PAK1 inhibited the phosphorylation of MEK and ERK in some BRAF WT cell lines (3 BRAF/NRAS WT, 1 NRAS mutant), which was associated with reduced cell viability. In a wider panel of NRAS mutant melanoma cell lines, PAK1 siRNA showed variable inhibition of growth. From a functional standpoint, PAK1 knockdown was associated with inhibition of both MEK1 and MEK2 signaling and suppression of cyclin D1 expression. Although PAK1 has been shown to phosphorylate MEK1 at S298 (a site that is lacking in MEK2), Ong et al. showed PAK1 knockdown to inhibit phosphorylation of MEK1 and MEK2 at S217/S221, suggesting the inhibitory effect was mediated at the level of CRAF (4).

Mutated BRAF is a bona fide therapeutic target in the majority of melanomas, and impressive clinical responses can be seen following treatment with small-molecule BRAF inhibitors such as vemurafenib and dabrafenib (14, 15). Other targeted therapies are being developed for other melanoma genotypes, with clinical activity recently being reported for single agent MEK inhibitors in the 15%–20% of patients whose melanomas harbor NRAS mutations (16). The findings of Ong et al. add PAK1 to a potential list of therapeutic targets for BRAF WT melanoma (4). In agreement with their genetic studies, Ong et al. showed the PAK1 inhibitor PF-3758309 to inhibit the phosphorylation of CRAF and MEK in PAK1 overexpressing (BRAF WT) melanoma xenografts, an effect associated with tumor stasis. Although efficacy was clearly seen following PAK1 inhibition, the inhibition of CRAF and ERK phosphorylation was incomplete, raising questions over the potential utility of this strategy in vivo (4). The evolving experience with vemurafenib in BRAF mutant melanoma has demonstrated
that near total pathway inhibition (>90%) is required for efficacy in patients, with recovery of MAPK pathway signaling common to most mechanisms of therapeutic escape reported (17). The dependence of PAK1 overexpressing BRAF WT melanomas upon CRAF/MAPK signaling may have identified another subgroup of patients who may benefit from the new generation of highly potent allosteric MEK inhibitors, such as trametinib and MEK-162, or even pan RAF inhibitors. These observations fit with earlier work demonstrating that some melanomas lacking BRAF-V600E mutations are dependent on CRAF for their survival and that knockdown/inhibition of CRAF leads to apoptosis associated with inhibition of phospho-BAD/Bcl-2 (18, 19). Whether the PAK1 overexpressing melanomas identified by Ong et al. are part of a larger group of tumors with CRAF dependency remains to be determined.

Appropriate caution should be shown when contemplating the testing of PAK1 inhibition in patients since its signaling may be important for leukocyte chemotaxis, as well as macrophage and T-cell activation (20). PAK1 signaling also appears to play a role in neuronal migration and polarization, and its absence may be associated with the onset of neurodegenerative diseases, suggesting that neurotoxicity may be a limiting side effect of its inhibition (21). As we continue to optimize targeted therapy in melanoma, resistance to single-agent therapies appears inevitable. The observation by Ong et al that PF-3758309 induced stasis and not tumor regression suggests that combination therapy may be required for PAK1-dependent melanoma. A PAK inhibitor, possibly not alone but in combination with a MEK inhibitor, might have clinical activity in BRAF WT melanoma, according to the authors’ data. The authors correctly assess that this constitutes an important unmet need. There is some evidence that BRAF resistance to current inhibitors may be reversed by the use of PAK inhibitors. There is already a wealth of evidence demonstrating that the PI3K/AKT/mTOR pathway is constitutively activated in most melanomas, with preclinical studies showing dual MAPK and PI3K/AKT inhibition to be more effective than single inhibitor therapy (22,23). In addition, other recent studies have shown BRAF WT melanomas (specifically melanomas with NRAS mutations) to be more susceptible to the combination of a MEK and a CDK4 inhibitor (24). It is expected that ongoing progress in this area will provide critical new information that will guide future clinical trial design and bring greater benefits to melanoma patients.

References

Notes
JW has been a paid consultant to Glaxo SmithKline Inc. and Genetech Inc, and he has been a member of a speaker's bureau for Genentech, Inc.

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