Anaphase-Promoting Complex Adaptor FZR1/CDH1 Blocks BRAF Signaling Both by Targeting BRAF for Proteolytic Degradation and by Disrupting BRAF Dimerization

Chao Zhang and Gideon Bollag

Summary: Blocking dimerization and stimulating protein degradation are two mechanisms known to inhibit BRAF activity. The study reported by Wan and colleagues identifies BRAF as a substrate of the APC/C^FZR1–ubiquitin–proteasome system. The interaction between FZR1 and BRAF also induces a conformational change that disrupts BRAF dimerization. These findings identify a dynamic interplay between FZR1 and BRAF with strong implications for cell-fate determination and the tumor suppressor role of FZR1. Cancer Discov; 7(4); 356–8. ©2017 AACR.

See related article by Wan et al., p. 424 (3).

Cell-cycle control occupies a central place in the complex code of regulations that govern the myriad processes inside our cells. The cellular machinery that precisely orchestrates the ordered series of events leading to high fidelity chromosome replication and segregation also safeguards the cells from deleterious effects that bring about catastrophes such as malignant transformation. Regulated protein degradation through ubiquitin-mediated proteolysis plays a prominent role in powering the cell-cycle clock (1). The irreversible nature of proteolysis helps ensure the directionality of cell-cycle progression. Two multisubunit RING ubiquitin E3-ligases, the SCF complex, which performs a variety of functions throughout the entire cell cycle, the APC/C is active mainly between metaphase and the end of the next G1 phase, with its tasks temporally segregated by the two adaptor proteins CDC20 and FZR1 (CDH1). Whereas APC/C^C^CDC20 initiates the metaphase–anaphase transition, APC/C^C^FZR1 promotes exit from mitosis and prevents premature entry into the S phase. A large number of mitotic regulators are degraded at the end of mitosis and kept in check by APC/C^C^FZR1. Because APC/C^C^FZR1 is required to maintain genomic stability (2), it ranks as a tumor suppressor: Loss of APC/C^C^FZR1 can cause uncontrolled proliferation, genomic instability, and tumorigenesis.

A new player is joining the FZR1 story (Fig. 1). FZR1 is now shown to negatively regulate BRAF via two very different mechanisms (3). It has been known for many years that the net effect of APC/C^C^FZR1 activity is typically cell-cycle progression. Now, BRAF is added to the FZR1 substrate list. Furthermore, the act of binding to BRAF results in a second, non-APC/C-dependent activity: disruption of BRAF dimerization. Interestingly, BRAF is the only member of the RAF family to be targeted by FZR1—ARAF and CRAF are spared. This sheds additional light on the tumor suppressor role of FZR1 and appears consistent with loss of FZR1 function in melanoma and other cancers. And like other tumor suppressor genes, loss of FZR1 in slowly cycling cells leads to senescence. In seeking to understand the role of FZR1 in senescence, Wan and colleagues (3) note the link to BRAF whose aberrant activation is linked to melanocyte senescence. Indeed, activating BRAF mutations are often found in benign nevi (4), thought to be senescent skin lesions originating from melanocytes, and Wan and colleagues find decreased levels of FZR1 in samples of human nevi. Pursuing this thread, the authors note a clear inverse correlation of FZR1 activity with BRAF protein levels. BRAF depletion depends on APC/C^C^FZR1 catalytic activity, and an FZR1 binding site was identified on BRAF. This so-called destruction-box (D-box) localizes to a surface loop in the BRAF catalytic domain. Discovery of a rare human cancer mutation, R671Q, in the BRAF D-box region strengthens this connection. Introduction of R671Q reduces FZR1 binding and BRAF degradation. Response to UV light provides another potentially significant link between BRAF and FZR1: UV-induced mutations accelerate BRAF-dependent oncogenesis, and UV separately induces FZR1 degradation and accumulation of FZR1 substrates.

Perhaps most surprising in the FZR1/BRAF story, however, is a direct interference of BRAF function that is independent of APC/C. Wan and colleagues show that FZR1 can disrupt BRAF dimerization (Fig. 1). Activation of the RAS protein induces BRAF dimerization, an essential event in stimulating the MAP kinase pathway. In a cell that is dependent on BRAF dimerization for ERK phosphorylation, elevated expression of FZR1 can block BRAF signaling. Again, this depends on an intact D-box domain implying direct binding between FZR1 and BRAF. Cancer cells that are activated by the most common BRAF oncogenic mutation, V600E, do not depend on BRAF dimerization and are not affected by depletion of FZR1 (although loss of FZR1 contributes to BRAF inhibitor resistance). By contrast, in cancer cells that depend on the dimerization-dependent BRAF mutation G466E, depletion of FZR1 enhances BRAF activity.
Further refinement of FZR1 activity proceeds through phosphorylation events (Fig. 1). Previous work has shown that CDK-dependent phosphorylation of FZR1 disrupts the interaction with APC/C (5). In the work currently under discussion, ERK-dependent phosphorylation of FZR1 causes similar loss of APC/C binding and thus prevents degradation of APC/C substrates such as BRAF. This would appear to be a positive feedback effect of BRAF activation. Coinhibition of ERK activity (using a BRAF or MEK inhibitor) and CDK4 activity (using a selective inhibitor) results in synergistic unmasking of APC/C activity and lower levels of the BRAF protein. And following up on the role of PTEN depletion in melanoma, Wan and colleagues also show that reduced FZR1 levels cooperate with PTEN loss in mouse melanocytes to activate BRAF signaling in vivo. These data support combination studies with kinase inhibitors to enable the tumor-suppressive effects of FZR1.

The FZR1/BRAF story may be quite relevant to melanoma and other cancers. In reviewing The Cancer Genome Atlas database, Wan and colleagues note that 23% of tumors harbor mutations or deletions in FZR1 or other APC/C subunit genes, whereas 80% of tumors harbor mutations or amplifications of BRAF or NRAS. Melanoma-derived FZR1 mutants show defective BRAF binding. As noted above, the most common BRAF mutants work as monomers and will not be significantly affected by FZR1 mutations. Nonetheless, resistance to BRAF and MEK inhibitors is often mediated by BRAF amplification and BRAF dimerization (6), so it will be important to monitor the effects of FZR1 loss on resistance to targeted therapy. The same FZR1/BRAF reciprocation may play a role in many additional cancers.

Although it is not surprising that protein stability plays an important part in the FZR1/BRAF story, the current specific mechanistic link to the APC/C ubiquitin system has not been anticipated in the literature. In the past, HSP90 has been shown to modulate the homeostasis of oncogenic BRAF (among a host of additional client proteins) on a more global level (7). The current study now adds a context-dependent interplay between the tumor suppressor FZR1 and the BRAF/MEK/ERK pathway, highlighting the intricacies of tumor development. The BRAF community should now include this new set of characters in its sights as better therapeutic options for patients with cancer are pursued. The plot thickens.

This is the first report linking BRAF to FZR1, and future studies will continue to unravel the intricacy and biological impact of this dynamic connection. For example, a structural understanding of how FZR1 binding to D-box motif modulates the dynamics of BRAF dimerization would illuminate new ways to pharmacologically inhibit RAS/BRAF signaling. Multiple interconnected effects on FZR1 activity and BRAF function are elicited by the CDK, ERK, and PI3K phosphorylation cascades. Efficacious coinhibition of these kinase pathways (8) could be explained by multiple events networked to FZR1/BRAF. Furthermore, deciphering the role of FZR1/BRAF in controlling the proliferation-senescence decision will enable new approaches to cancer treatment that promote the senescence program in tumor cells (9).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online April 3, 2017.
REFERENCES

Anaphase-Promoting Complex Adaptor FZR1/CDH1 Blocks BRAF Signaling Both by Targeting BRAF for Proteolytic Degradation and by Disrupting BRAF Dimerization

Chao Zhang and Gideon Bollag


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><a href="http://cancerdiscovery.aacrjournals.org/content/7/4/356">http://cancerdiscovery.aacrjournals.org/content/7/4/356</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cited articles</th>
<th>This article cites 9 articles, 3 of which you can access for free at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><a href="http://cancerdiscovery.aacrjournals.org/content/7/4/356.full#ref-list-1">http://cancerdiscovery.aacrjournals.org/content/7/4/356.full#ref-list-1</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E-mail alerts</th>
<th>Sign up to receive free email-alerts related to this article or journal.</th>
</tr>
</thead>
</table>

| Reprints and Subscriptions | To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org. |

| Permissions | To request permission to re-use all or part of this article, use this link [http://cancerdiscovery.aacrjournals.org/content/7/4/356](http://cancerdiscovery.aacrjournals.org/content/7/4/356). Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site. |