IN THE SPOTLIGHT

RNF2 E3 or Not to E3: Dual Roles of RNF2 Overexpression in Melanoma

Joshua C. Black and Johnathan R. Whetstine

Summary: RNF2/RING1B is amplified and overexpressed in numerous tumors and contributes to tumorigenicity; however, the biologic importance is poorly understood. Surprisingly, the role of RNF2 in tumorigenesis and invasion can be separated into catalytically independent and catalytically dependent processes. Cancer Discov; 5(12); 1241–3. ©2015 AACR.

See related article by Rai et al., p. 1314 (5).

RNF2/RING1B and its highly homologous counterpart RING1A are interchangeable core subunits of the Polycomb Repressive Complex 1 (PRC1; refs. 1, 2). RNF2 and RING1A are members of the RING finger family of proteins and function as E3 ubiquitin ligases for monoubiquitination of histone H2A at lysine 119 (H2AK119ub). H2AK119ub helps mediate Polycomb Group (PcG) silencing by promoting chromatin compaction and antagonizing histone marks correlated with transcriptional activation. H2AK119ub is required for most, but not all, PRC1 transcriptional silencing activities (1, 2).

RNF2 is ubiquitously expressed in human tissues and is amplified or overexpressed in many human tumors (3). The biologic consequences of this overexpression remain elusive; however, RNF2 was recently identified in a signature for invasive melanomas (4). Therefore, Rai and colleagues set out to ascertain a role for RNF2 in melanoma tumorigenesis and metastasis (5). Higher RNF2 expression levels correlated with progression and metastasis in melanoma. Furthermore, elevated RNF2 levels correlated with worse overall survival, indicating that RNF2 levels may have prognostic value in melanoma.

Overexpression of RNF2 was sufficient to promote invasion and metastasis in melanoma cell lines, whereas loss of RNF2 from high-expressing cell lines reduced metastatic potential (Fig. 1). RNF2 overexpression also promoted soft-agar growth and tumorigenic potential of melanoma cell lines. However, a surprising result came when it was revealed that only the proinvasion properties of RNF2 required the catalytic activity of the E3 ligase RING domain, whereas the pro-oncogenic promotion of proliferation and anchorage-independent growth was catalytically independent (Fig. 1; ref. 5).

The authors started to reconcile the disparity in catalytic activity requirements through genomic and transcriptomic profiling of RNF2-overexpressing cells. RNF2-bound and RNF2-regulated transcripts were split between repressed transcripts (47%), which represent the classic repressive function of RNF2, and upregulated transcripts (53%), representing a new possible transcriptional activation function (5). Transcripts repressed by overexpression of RNF2 were enriched in genes regulating transcription and nucleotide synthesis. However, the most robust gene expression changes were associated with TGFβ signaling, which is an established regulator of invasion and metastasis. However, most robust gene expression changes were associated with TGFβ signaling, which is an established regulator of invasion and metastasis. Rai and colleagues demonstrated that LTBP2, which is a member of the latent TGFβ binding proteins, is transcriptionally repressed in a catalytically dependent manner by RNF2 overexpression. The LTBP2 promoter was directly bound by RNF2 in overexpressing cells with a concomitant increase in H2AK119ub. In agreement with these observations, knockdown of LTBP2 resulted in the upregulation of TGFβ targets (ID1-3; ref. 5). Consistent with a role for increased TGFβ signaling, RNF2-mediated invasion was suppressed by the TGFβ signaling inhibitor LY2157299 (Fig. 1).

Taken together, these results suggest that increased TGFβ signaling by inappropriate silencing of TGFβ regulators by H2AK119ub potentiates melanoma cell invasion and metastasis. One important question arising from this work is: Why is RNF2 targeted to LTBP2 only in cells highly expressing RNF2? Typically, RNF2 and PRC1 are recruited to targets through association of the chromodomain of the CBX family members of PRC1 with histone 3 lysine 27 trimethylation (H3K27me3; refs. 1, 2). However, it is unclear if other PRC1 components are required for the silencing of LTBP2 or if PRC2 or H3K27me3 are required as well. None of the other PRC1 or PRC2 components were revealed in the prometastatic signature for melanoma (4). This could reflect a property unique to RNF2. Therefore, it will be interesting to see what is necessary for RNF2 recruitment to targets specified by overexpression.

Like RNF2, RING1A can also create H2AK119ub (1, 2). Interestingly, RING1A did not emerge from the prometastatic melanoma screen. It remains unclear if overexpression of RING1A also promotes invasion or tumorigenesis (Fig. 1). Rai and colleagues clearly demonstrate that in melanoma RNF2 is necessary and sufficient for suppression of LTBP2 and modulating invasion and metastasis (5); however, it is
possible that RING1A could potentiate similar effects in other tissue or tumor types. It will be important to determine the unique and overlapping targets for RNF2 and RING1A and what specifies these differences.

In addition to ubiquitinating H2AK119, RNF2 can also monoubiquitinate H2A.Z at lysines 120, 121, and 125 (6). It is possible that H2A.Z may also be a target for ubiquitination by RNF2 overexpression in melanoma (Fig. 1). In agreement with this possibility, H2A.Z isoforms are overexpressed in melanoma and contribute to the cancer phenotype (7). Although H2A.Z incorporation has generally been correlated with transcriptionally active chromatin, monoubiquitinated H2A.Z localizes to heterochromatin and the inactive X-chromosome, suggesting it is a mark for transcriptional silencing or repression (8). Therefore, it may be important to silence increased H2A.Z at tumor suppressor loci through ubiquitination, while leaving oncogenes transcriptionally active.

H2AK119ub may be important in other malignancies as well. Recent work from the Green laboratory has uncovered a new enzyme capable of monoubiquitination of H2AK119, TRIM37 (9). TRIM37 is amplified and overexpressed in breast cancers with reduced levels of RNF2. TRIM37 associates with PRC2 and mediates ubiquitination and silencing of tumor suppressors. Thus, at least two enzymes mediating H2AK119ub are amplified and overexpressed in tumors. It may be worthwhile to identify ways to upregulate the H2AK119 deubiquitinasenase, USP10, or identify agonists to reduce H2AK119 levels (6). It may be beneficial to explore inhibiting H2AK119ub in order to reduce tumor invasion and metastasis (Fig. 1).

Rai and colleagues also uncovered how the catalytic-independent functions of RNF2 can contribute to oncogenesis (5). Fifty-three percent of genes bound by RNF2 were upregulated upon overexpression, which suggested a potential transcriptional activator role for RNF2. RNF2 was recently demonstrated to exist in at least one complex distinct from PRC1 in mouse cells. This complex contained UTX/KDM6A, p400, and MLL2, suggesting a transcriptional coactivator function (10). Consistent with these observations, Rai and colleagues demonstrated that RNF2 can become phosphorylated in an MEK1-dependent manner, which enables recruitment of UTX/KDM6A and p300 to activated target genes, such as Cyclin D2 (CCND2). Depletion of CCND2 partially rescued proliferation, anchorage-independent growth, and tumorigenesis, which indicates at least part of the oncogenic potential of RNF2 overexpression is mediated through transcriptional activation of CCND2 (5). It is possible that transcriptional activation of other oncogenes through RNF2-mediated activation also contributes to the pro-oncogenic potential of RNF2 overexpression.

Similar to the repressed genes, it is unclear how overexpressed RNF2 is targeted to the transcriptionally activated genes. One possibility is the p300 bromodomains facilitate recruitment to specific acetylated residues. Alternatively, both UTX and p300 are known to associate with DNA-binding transcriptional activators and, in turn, facilitate recruitment
of RNF2 to certain targets (10). Finally, RNF2 itself may directly interact with transcription factors in a MEK1 phosphorylation-dependent manner. Understanding the recruitment mechanisms may enable design of therapeutics that could prevent activation of genes promoting oncogenesis.

Another important question is how and where does RNF2 become phosphorylated? One intriguing possibility is that phosphorylation by MEK1 can occur on RNF2 in the PRC1 complex, which would lead to its dissociation and formation of the new transcriptionally active complex with UTX. If this were able to occur on chromatin at PcG repressed genes, it could trigger a switch to gene activation. This phospho switch in RNF2 function could serve as a cue during differentiation to trigger derepression of lineage-specific bivalent genes. It will be interesting to determine how this posttranslational modification of RNF2 affects Polycomb complex assembly and recruitment and whether this signaling cascade is a normal biologic mechanism for regulating silencing or gene activation during development and differentiation.

Finally, the work from Rai and colleagues suggests that some new potential therapeutic combinations may be effective in melanoma and possibly other solid malignancies. First, catalytic inhibitors of RNF2 may be able to help abrogate metastasis of primary melanomas and help reduce tumor burden and metastatic disease (Fig. 1). Second, MEK signaling inhibitors could be beneficial in melanomas with increased RNF2 levels. Combinations of MEK inhibitors and RNF2 catalytic inhibitors may be able to reduce both metastatic and primary tumor burden. Third, RNF2 and p300 inhibitors may also work well together in treating melanoma. Finally, it will be interesting to see if RNF2, TRIM37, or RING1A drive similar metastatic and oncogenic programs outside of melanoma and breast cancer and whether any of these potential therapies will be important in those settings.

Disclosure of Potential Conflicts of Interest
J.R. Whetstine is a consultant/advisory board member for Qsonica. No potential conflicts of interest were disclosed by the other author.

Grant Support
J.R. Whetstine was supported by NIH R01GM097360 and is the Tepper Family Massachusetts General Hospital Research Scholar.

Published online December 4, 2015.

REFERENCES