

Essential signaling in *NF2* loss-related tumours: the therapeutic potential of CRL4^{DCAF1} and mTOR combined inhibition

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Neurofibromatosis 2 (*NF2*) was first identified as the tumor suppressor gene mutated in *NF2* hereditary cancer syndrome (1). In this syndrome, patients develop multiple tumors of the nervous system including schwannomas, meningiomas and ependymomas that often require treatment with surgery or radiotherapy. While inherited mutations in *NF2* leading to *NF2* syndrome are relatively uncommon (with an incidence of approximately 1 in 25,000), somatic *NF2* mutations are also observed in sporadic tumors (2). Specifically, *NF2* gene inactivation has been reported in a significant proportion of sporadic meningiomas, vestibular schwannomas and malignant mesotheliomas (3-6). Furthermore, the protein product of the *NF2* gene, Merlin, has been found to antagonize tumor initiation and/or progression in breast, colorectal, prostate, hepatobiliary and medullary thyroid cancers as well as in glioblastoma and melanoma (7). Therefore, there is tremendous interest in understanding the precise roles that *NF2* gene loss and the protein Merlin play within diseased and normal cells, respectively, in order to develop new therapies for cancer treatment.

Merlin has multiple functions within healthy cells that can be distinguished by subcellular localization. Critically, there is a lack of consensus in the literature regarding which of these interactions are essential for *NF2* gene loss-related tumorigenesis. As a close relative to the Ezrin-Radixin-Moesin (ERM) family of proteins, Merlin associates with the inner leaflet of the plasma membrane where it regulates adherens junction stability and augments numerous

signaling pathways including EGFR, Ras, Rac, mTOR, FAK-Src, PI3K, Hippo and Lin28B signaling (8-12). However, over the past two decades, multiple groups have suggested that Merlin acts as a tumor suppressor within the nucleus and, in 2010, Li *et al.* provided compelling evidence supporting this notion (13-16). In their work which was published in *Cell*, Li *et al.* demonstrated that Merlin translocates into the nucleus to inhibit the cullin-RING E3 ubiquitin ligase CRL4^{DCAF1}. In a subsequent *Cancer Cell* publication, Li *et al.* characterized the signaling downstream of CRL4^{DCAF1} in Merlin-deficient cells (17). CRL4^{DCAF1} directly ubiquitinylates and destabilizes the Hippo pathway tumor suppressor kinases LATS1 and LATS2 in the nucleus. This inhibition of LATS1/2 activates the Hippo pathway effector and transcriptional co-activator YAP leading to the transcription of genes involved in tumorigenesis. Based on these data, Li *et al.* proposed that Merlin suppresses tumorigenesis from within the nucleus (and not from the cell cortex) through a Merlin-CRL4^{DCAF1}-LATS1/2-YAP signaling axis. However, whether this signaling might provide useful therapeutic targets for the treatment of *NF2*-inactivated cancers was unclear.

In a recent paper published in *Molecular Cancer Therapeutics* (18), Cooper *et al.* set out to determine whether CRL4^{DCAF1} might be an efficacious therapeutic target for blocking *NF2* gene loss-related tumorigenesis. In their paper, the authors used a small molecule drug (MLN4924) to indirectly block CRL4^{DCAF1} ubiquitin ligase activity by targeting its upstream activator NEDD8-activating

enzyme (NAE). The authors first confirmed that MLN4924 treatment augments the signaling identified by their previous studies. Indeed, MLN4924 suppressed CRL4^{DCAF1} activity and LATS1/2 ubiquitinylation *in vitro*. Moreover, MLN4924 induced inhibitory YAP phosphorylation at serine 127 in FC-1801 mouse *Nf2*-mutant schwannoma cells as well as in the human *NF2*-mutant mesothelioma cell line, Meso-33. In functional assays, MLN4924 reduced the proliferation of both *NF2*-mutant and *NF2*-wild type cells. However, *NF2*-mutant cells appeared to be more sensitive to growth inhibition by MLN4924 than their wild type counterparts. To explore the anti-proliferative effects of MLN4924 *in vivo*, Cooper *et al.* established tumor xenografts in immunocompromised mice with *NF2*-mutant cell lines VAMT and Meso-10. Tumor growth was compared between mice administered MLN4924 or vehicle. MLN4924 diminished the rate of tumor growth but could not halt growth entirely or cause tumor regression.

Given that MLN4924 showed limited preclinical activity as a monotherapy, Cooper *et al.* postulated that MLN4924 might hold greater therapeutic value in combination with other drugs. The authors first exploited the fact that pharmacological inhibition of NAE with MLN4924 not only represses the activity of CRL4^{DCAF1} but also blocks other cullin-RING ligases including those involved in DNA damage responses (19). Therefore, they reasoned that collateral inhibition of these ligases by MLN4924 might sensitize cells to DNA-damaging chemotherapies. As expected, combined MLN4924, pemetrexed and cisplatin treatment reduced VAMT and Meso-10 *in vivo* tumor growth more dramatically than MLN4924 on its own. However, even this combination of drugs could not abolish tumor growth completely. The authors proceeded to develop a more targeted strategy for combination therapy based on the signaling they observed in their *NF2*-mutant cell lines. In their molecular experiments, Cooper *et al.* observed that MLN4924 treatment had no effect on mTORC1 activity or its downstream signaling through S6 in *NF2*-mutant cells. Thus, *NF2* gene loss likely activates this pathway independent of CRL4^{DCAF1}. To investigate whether mTOR signaling sustains *NF2*-mutated cancer cells during CRL4^{DCAF1} blockade, Cooper *et al.* treated mesothelioma tumor xenografts with MLN4924 and GDC-0980 (a dual mTOR/PI3K inhibitor). Combined MLN4924/GDC-0980 treatment completely abolished tumor growth *in vivo* for four weeks. From this work, Cooper *et al.* conclude that both CRL4^{DCAF1} and mTORC1 signaling are essential oncogenic pathways in *NF2*-inactivated cancers.

Over the past several decades, a multitude of functions for Merlin have been demonstrated in the literature. However, which of these activities are critical for driving tumorigenesis in *NF2*-mutated cells has been unclear. In their study, Cooper *et al.* pharmacologically inhibit two proteins (CRL4^{DCAF1} and mTORC1) that are aberrantly activated in Merlin-deficient cells to clarify the signaling that drives tumor growth. This study provides novel insights into the key tumor suppressor functions of Merlin and raises interesting questions for future studies. Most significantly, Cooper *et al.* have provided strong preclinical data showing that combined inhibition of CRL4^{DCAF1} and mTORC1 may be a good therapeutic strategy for the treatment of *NF2*-inactivated cancers.

While Cooper *et al.* have generated exciting preclinical data supporting this proposition, several critical issues should be addressed prior to applying these findings in a clinical setting. First, it will be crucial that future studies investigate the durability of responses to MLN4924/GDC-0980. In their study, Cooper *et al.* observed inhibition of tumor growth *in vivo* by these agents for only four weeks. Thus, it is unclear whether the treatment applied truly halts disease progression over the long-term. Second, given that MLN4924 targets multiple cullin-RING ubiquitin ligases, it is still unclear whether the anti-proliferative effects of MLN4924 in *NF2*-mutated cells are due to specific inactivation of CRL4^{DCAF1}-LATS1/2-YAP signaling. The contributions of CRL4^{DCAF1} and YAP to the response to MLN4924 could be tested relatively easily by examining whether genetic knockout cells for each of these proteins are rendered less susceptible to MLN4924 treatment. Third, it may also be worthwhile to explore whether there are alternative strategies that can be used to achieve better specificity for targeting CRL4^{DCAF1} particularly since mechanisms for resistance to MLN4924 have already been reported in the literature (20). The signaling downstream of CRL4^{DCAF1} may offer alternative opportunities for therapeutic intervention. YAP can also be targeted therapeutically and Furukawa *et al.* have recently proposed that Merlin can directly regulate the nuclear export of YAP and its paralog TAZ (21). Therefore, YAP may hold promise on its own as a therapeutic target. It will be interesting for future studies to test whether anti-YAP/TAZ monotherapy or combined inhibition of YAP/TAZ and mTORC1 represent therapeutic targets for *NF2*-inactivated malignancies in the future.

Finally, it should also be noted that there have been other agents for treating *NF2*-inactivated tumors. In fact, the off-

label use of anti-VEGF antibodies for treating patients with *NF2* syndrome has become more common over the past few years (22). Other targeted therapies (including inhibitors of EGFR, PDGFR, VEGFR and c-kit) have shown promise in preclinical and clinical studies (6,23). Thus, it will also be important to explore whether these treatments combined with CRL4^{DCAF1} or/and mTORC1 inhibition hold value for more effectively treating *NF2* gene loss-related malignancies in the future.

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Footnote

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