The Need for Neddylation: A Key to Achieving NED in Uveal Melanoma

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The ability of uveal melanoma cells to enter and exit dormancy plays a fundamental role in the development of metastatic disease. Neddylation blockade is a promising strategy to prolong tumor dormancy via impaired angiogenesis and prevent the establishment of metastases via elimination of cancer stem-like cells. Clin Cancer Res; 24(15); 3477–9. ©2018 AACR.

In this issue of Clinical Cancer Research, Jin and colleagues (1) demonstrate that neddylation blockade inhibits uveal melanoma (UM) tumor growth and hepatic metastases via repression of cancer stem-like cells (CSC) and angiogenesis.

UM is a rare, biologically distinct subset of melanoma characterized by the development of clinically apparent metastases years after treatment of the primary tumor. Despite excellent rates of local control with surgery or radiotherapy, nearly 50% of patients with UM succumb to metastatic disease within 15 years of initial diagnosis. The extended latency period between diagnosis and distant disease implies early dissemination of tumor cells and the ability for UM cells to enter a state of dormancy, becoming quiescent, but not senescent. Mathematical modeling of cell doubling times suggests that dormant UM cells are present even before the primary ocular tumor is found (2), consistent with the detection of circulating tumor cells in the majority of UM patients with only localized disease at the time of diagnosis (3). The persistence of these circulating malignant cells in the absence of overt metastases points to the ongoing survival of occult, dormant cells or microscopic foci. However, not every patient with UM develops distant disease, begging the question of why, when, and how these cells begin to proliferate and establish clinically significant metastases. Given the dearth of effective treatments for metastatic UM, strategies to maintain tumor dormancy and/or drive dormant cells into senescence present promising therapeutic approaches.

The ubiquitin-proteasome system (UPS) plays an integral role in maintaining cellular homeostasis through targeted protein degradation. The cullin-RING ligases (CRL) represent the largest family of E3 ligases and are responsible for the ubiquitination of roughly 20% of UPS-degraded proteins. CRLs control the turnover of key substrates involved in processes relevant to tumorigenesis, including cell-cycle progression, DNA repair, apoptosis, and signal transduction pathways, thus providing a rationale for targeting CRLs as an anticancer strategy. CRL activation requires the covalent addition of NEDD8, a ubiquitin-like protein, to the cullin protein in a reaction known as neddylation (Fig. 1). Inhibition of NEDD8-activating enzyme (NAE) or other components of the neddylation pathway disrupts CRL-mediated ubiquitination of key players involved in tumor growth and survival. MLN4924 (pevonedistat) is a first-in-class, potent small-molecule NAE inhibitor with preclinical antitumor activity against a number of solid and hematologic malignancies. Postulated mechanisms include induction of apoptosis (accumulation of CD1 and NOXA), autophagy (accumulation of DEPTOR and HIFα leading to mTOR inactivation), senescence (p21 dependent), and increased sensitization to chemotherapy and radiation. In the present study, Jin and colleagues show that expression of the neddylation enzymes NAE1, UBA3, and UBC12 are upregulated in UM cells compared with normal retinal pigment epithelial cells (1). Moreover, NAE expression correlates with primary tumor basal diameter and thickness, factors associated with increased metastatic risk. The authors proceed to show that MLN4924 inhibits UM cell growth via activation of apoptosis and the DNA damage response, triggered predominantly by formation of double-stranded DNA breaks. In vivo efficacy of MLN4924 was confirmed using a NOD-SCID mouse xenograft model, and tumor analysis showed inhibition of the neddylation pathway with increased levels of the CRL substrates p21 and p27.

More intriguing are the observed effects of NAE inhibition on angiogenesis, a process implicated in tumor dormancy, and cellular senescence. Genome-wide transcriptional analysis of dormant and rapidly proliferating human tumors found differential upregulation of antiangiogenic genes in dormant cells. In the present study, the authors show that MLN4924 blocks angiogenesis by impairing VEGF-C secretion, resulting in reduced microvascular density of UM hepatic metastases (1). Conditioned medium derived from treated cells also display diminished capacity to promote migration and neovascularization. Preclinical studies demonstrate that MLN4924 triggers an irreversible, p21-dependent senescence associated with sustained activation of the DNA damage response. In fact, notably increased levels of p21 are observed in UM tumors harvested from MLN4924-treated mice. Thus, NAE inhibition may not only contribute to sustained tumor dormancy via impaired angiogenesis but also eradicate dormant tumor cells, which are generally not susceptible to conventional cytotoxic agents, by actuating cellular senescence.

How tumor cells subsequently exit dormancy to undergo extravasation and colonization of distant organs is unclear, but...
it stands to reason that these cells must acquire certain stem-like properties to reinitiate and sustain tumor growth within a new microenvironment. Indeed, a population of stem-like cells with enhanced self-renewal and proliferative capacities has been identified in UM cell lines (4). Further corroborating the role of CSCs in UM pathogenesis is the prognostic significance of BAP1 (BRCA1-associated protein 1) mutations, found in approximately 47% of primary UM and 84% of metastatic UM cases, which are strongly associated with increased metastatic risk. Loss of BAP1 abrogates melanocytic differentiation and leads to acquisition of a class 2 gene expression profile, which confers multipotent, stem cell–like properties (5, 6). Thus, we can surmise that BAP1 mutations mechanistically drive metastasis by promoting the formation of CSCs. Jin and colleagues show that MLN4924 inhibits self-renewal and maintenance of CSCs in UM cell lines via increased degradation of the zinc-finger transcription factor Slug, a known regulator of epithelial-to-mesenchymal transition (1). By eliminating CSCs, MLN4924 may prevent UM cells from exiting dormancy and establishing overt metastases.

Although MLN4924 monotherapy appears to have limited to modest activity in the completed phase I trials (7–10), ongoing studies are investigating MLN4924 in combination with various cytotoxic and epigenetic modifying agents. In a phase Ib study in elderly, treatment-naïve AML patients unfit for standard induction chemotherapy, MLN4924 plus azacitidine achieved a promising 50% response rate and an 8.3-month median duration of remission (11). The findings presented by Jin and colleagues (1) further support the use of combinatorial strategies targeting apoptosis and the DNA damage response pathway. In the present study, MLN4924 induces apoptosis in UM cells and warrants further preclinical investigation. The induction of DNA damage, apoptosis, autophagy, and senescence, all of which inhibit tumor growth.
double-stranded DNA breaks additionally points to a possible role for combined PARP inhibition. Finally, the findings by Jin and colleagues suggest that NAE inhibition may be more effective in the adjuvant setting. The development of metastatic disease in UM revolves around the early dissemination of tumor cells, their initial maintenance in a dormant state, and the eventual reawakening of cells with stem-like properties capable of reinitiating growth at distant sites. NAE inhibition targets several of these key processes by prolonging tumor dormancy, eradicating dormant UM cells by actuating senescence, and preventing the establishment of clinically significant metastases via elimination of CSCs.

In summary, neddylation blockade presents a novel treatment strategy for inhibiting tumor growth and metastasis in UM, an aggressive malignancy with no effective therapies in the metastatic setting. Promising combinatorial approaches targeting pathways involved in apoptosis, DNA damage response, and epigenetic modification should be further explored.

References
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