AUTHOR'S VIEW

Uncovering a novel pathway for p16 silencing: Therapeutic implications for lung cancer

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ABSTRACT

A key step during onset of most cases of non-small cell lung cancer (NSCLC) is the loss of the tumor suppressor p16^{INK4a} (best known as p16), commonly due to promoter hypermethylation. We recently reported a novel regulatory pathway involving E6-associated protein and cell division control protein 6, which provides a methylation-independent mechanism for p16 silencing in patients with a particularly aggressive form of NSCLC.

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Commentary

Non-small cell lung cancer (NSCLC), constituting 80% of all lung cancers, is the leading cause of cancer-related mortality worldwide. Despite important advances in treatment, the disease is rarely curable and prognosis is poor, with a dismal overall 5-year survival rate of only 20%. This highlights the importance of enhancing our knowledge of the underlying molecular events of NSCLC to develop rationally designed methods for early detection and prevention, as well as to discover novel therapeutics approaches for treatment of this disease.

The most common genetic event leading to NSCLC is loss of the tumor suppressor p16^{INK4a} (best known as p16), occurring in ~70% of patients. P16, together with the key tumor suppressors p14^{ARF} and p15^{INK4b}, is encoded in the *INK4/ARF* locus, one of the genomic regions most commonly affected in human cancers. Consistent with the role of p16 as a tumor suppressor, low p16 levels are associated with poor prognosis and therapeutic resistance of NSCLC patients. Understanding the mechanisms leading to p16 loss in NSCLC is central to restoring its tumor suppression and improve patient prognosis. Several mechanisms have been defined to explain the loss of p16 in NSCLC: epigenetic silencing of the entire *INK4/ARF* locus, genetic deletions and mutation of the gene encoding p16 (*CDKN2A*), and *CDKN2A* promoter hypermethylation; this last mode being the most common (~37% of the patients) (Fig. 1). Together, these account for the loss of p16 in two-thirds of NSCLC cases, but the molecular explanation for the remaining cases is unknown. In our recent study published in *Science Signaling* we addressed, at least in part, this gap in knowledge.¹ We unraveled a novel mechanism for the reduction of p16, involving the E3 ubiquitin ligase and transcriptional cofactor,

E6-associated protein (E6AP), which we found to be lowly expressed in certain NSCLCs.

Our analysis identified E6AP as a positive regulator of the *INK4/ARF* locus. Mechanistically, we showed that E6AP controls this locus by reducing the ability of E2F transcription factor 1 (E2F1) to bind to the *cell division control protein 6* (*CDC6*) promoter. *CDC6* is a repressor of the *INK4/ARF* locus, and consequently low E6AP levels relieve this repression by *CDC6* (Fig. 1). The extent by which E2F1 activity was inhibited by E6AP was comparable to that achieved by retinoblastoma protein (RB1, best known as pRb), the key inhibitor of E2F1 during the cell cycle. However, this action of E6AP is independent of pRb.

We demonstrated the clinical relevance of the E6AP-*CDC6*-p16 axis by analyzing NSCLC human samples. This analysis revealed that only a small percentage of patients with the E6AP-low/*CDC6*-high/p16-low expression profile exhibited p16 methylation. This suggested that the E6AP-*CDC6*-regulatory axis represents a methylation-independent mechanism for p16 silencing in NSCLC. Importantly, NSCLC patients with E6AP-low/*CDC6*-high/p16-low expression profile have particularly poor survival rates, rendering this expression profile as a potential prognostic biomarker. Interestingly, our analysis demonstrated that low E6AP levels correlate with poor prognosis of patients with lung adenocarcinoma but not squamous cell carcinoma.

In search for an explanation for the specificity of E6AP to adenocarcinoma we noticed a strong association between the E6AP-low/*CDC6*-high/p16-low expression profile and activating mutations of the *KRAS* oncogene. These are the most frequent oncogenic alterations in lung adenocarcinomas, but rarely identified in squamous cell carcinomas. A strong link

Figure 1. Mechanisms involved in p16 loss in non-small cell lung cancer (NSCLC) and potential therapeutic approaches to restore p16 expression. The diagram on the left depicts how low abundance of E6-associated protein (E6AP) promotes lung tumorigenesis by silencing p16 in a methylation-independent manner: in the context of low or lost expression of E6AP, E2F transcription factor 1 (E2F1) is free to stimulate *cell division control protein 6* (*CDC6*) expression, which represses the *INK4/ARF* locus and contributes to p16 loss.

between KRAS activating mutations and p16 is also found in pancreatic carcinoma (95% KRAS mutations and 90% p16 loss), and colorectal cancer (45% KRAS mutations and 70% p16 loss).^{2,3} Activated KRAS promotes senescence by inducing *INK4/ARF*.⁴ Therefore, loss of p16 is necessary to overcome senescence allowing tumor progression. This possibility is supported by findings from Bennecke et al.⁵ showing that deletion of *INK4/ARF* in oncogenic KRAS-expressing mice prevents senescence and leads to an aggressive form of colorectal cancer. Therefore, we hypothesize that low E6AP levels cooperate with KRAS to bypass oncogenic-induced senescence in lung adenocarcinoma patients. It would be interesting to examine whether the E6AP levels are also low in other KRAS-driven cancers to test the extent of our hypothesis. Consistent with this notion, we previously published that cellular senescence induced by activated KRAS is compromised by loss of E6AP.⁶ In this context, it would be of interest to explore the involvement of the E6AP-*CDC6* axis in p16 loss in other KRAS-driven cancers.

Our study opens the opportunity to explore the therapeutic potential of targeting the E6AP/*CDC6*/p16 axis to restore p16 levels to treat NSCLC patients with adenocarcinoma. Over the past decade, identification of *epidermal growth factor receptor* mutations and *anaplastic lymphoma kinase* fusions has led to advances in the treatment of NSCLC through the use of targeted therapies. However, despite initial responses, tumors typically develop acquired resistance to these agents. The frequent silencing of tumor suppressors like p16 by hypermethylation opens the possibility of using epigenetic-based therapies in NSCLC. Indeed, DNA methyltransferases inhibitors have been very successful in the treatment of hematological malignancies. Encouragingly, combination of epigenetic-targeted therapy, inhibiting both DNA methyltransferases and histone deacetyltransferases, act synergistically to restore the expression of tumor suppressors, including p16, leading to tumor response in preclinical models of lung cancer.⁷ Importantly, a phase I/II trial combining the DNA methyltransferase inhibitor azacitidine and the histone deacetylase inhibitor entinostat in patients with recurrent metastatic NSCLC showed that demethylation of *CDKN2A* was associated with improved progression-free and overall survival.⁸

While the above therapeutic possibilities are relevant to patients with hypermethylated *CDKN2A*, our findings open the

possibility to design therapies to restore p16 expression in patients independent of hypermethylation. One option of countering the deregulated E6AP/*CDC6*/p16 axis is by inhibiting *CDC6*. An exciting possibility to reduce *CDC6* expression is by targeting monocytic leukemia zinc finger protein (MOZ), a member of the MYST family of histone acetyltransferases. MOZ was recently shown to induce *CDC6* expression and consequently repress the *INK4/ARF* pathway.⁹ Importantly, the development of small molecule inhibitors of MOZ is currently under way.¹⁰

In summary, our paper by Gamell et al. describes a novel regulatory pathway that provides a molecular explanation for the loss of the tumor suppressor p16 in adenocarcinoma, the most common sub-type of lung cancer. This opens an exciting possibility for the development of precision medicine therapies to restore p16 expression and consequently improve outcomes of lung cancer patients.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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