Dual-targeting of MEK and CDK4/6 in KRAS-mutated non-small cell lung cancer

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Received: May 27, 2016
Published online: July 05, 2016

Discovering new ways to modulate the responsiveness of non-small cell lung cancer (NSCLC) to treatment represents one of the forefronts of cancer research. KRAS mutations, present in roughly 30% of NSCLCs, lead to increased levels of cellular proliferation and decreased responsiveness to treatment modalities. The present study examined the RB/p16/CDK4 pathway as a way to modulate the responsiveness of these cell lines. The diverse downstream effects of these pathways suggest that molecular profiling of NSCLCs may assist clinicians and researchers to predict the effectiveness in the application of particular therapeutics.

Keywords: NSCLC; KRAS; lung cancer; MEK; RB; CDK4


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Lung cancer is the leading cause of cancer-related mortality in the United States, with an estimated annual mortality of 160,000 [1]. Non-small cell lung cancers (NSCLCs), which account for roughly 85% of all lung cancers, generally present in patients at advanced stages and require aggressive treatment modalities [2]. Since the mid-2000s, scientists have studied targeted therapies for NSCLC as potential alternatives to conventional chemotherapy [3-7].

The epidermal growth factor receptor (EGFR) and its downstream effectors represent an area of research. Specifically, the RAS-RAF-MEK-ERK pathway downstream of EGFR and its multiple potential targets (Figure 1). KRAS mutations are found in up to 30% of NSCLCs; they involve the mitogen-activated protein kinase (MAPK) pathway for cellular. NSCLCs with KRAS mutations have diverse downstream effectors [8-10]. Thus, targeted therapies may be used to treat various components.

MEK1/2 are protein kinases present in the proliferative pathway involving RAS-RAF-MEK-ERK (Figure 1). Previous studies demonstrated that MEK inhibitors (MEKi) have efficacy in treating KRAS mutant NSCLCs [11, 12]. Moreover, MEKis have little cross reactivity when used in sensitive NSCLC cell lines [13-15]. Our study examined how KRAS-mutant NSCLCs could be resensitized to this MEKi. One of the challenges research must address is characterizing the physiologic responses of drugs outside of their main effects.
A recent study done on colon cancer demonstrated that trametinib, a MEKi, had the ability to reactivate retinoblastoma protein through an unknown pathway [16, 17]. Additionally, a comprehensive genetic study using the TCGA was completed in order to determine the mutation profiles of KRAS-mutant NSCLCs in our experimentation. The data demonstrated a p16 mutation was associated with worse outcomes. Interestingly, we also found that the 6 cell lines used in our experiments that were resistant to MEKi were also p16 mutants. Given this finding in conjunction with previous research implicating RB pathway proteins in MEKi activity, we devised subsequent experiments to examine these interactions.

As shown in Figure 1, MEK1/2 kinases play an integral role in the RAS-RAF-MEK-ERK pathway involved in cellular growth and proliferation [18]. Mutated KRAS constitutively activates downstream effectors such as cyclin D1 and ERK1/2 [19]. Since previous studies have demonstrated the reactivation of RB protein due to MEKi and our cell lines resistant to MEKi were deficient in p16 protein, we decided to reintroduce a competent p16 protein via p16 plasmid vectors in deficient cell lines. Surprisingly, we found that these cell lines then became resensitized to MEKi.
Our results support the hypothesis that in a functionally normal cell, there remains a balance between p16, CDK4/6-cyclin D1, RB and E2F transcription factors in modulating the cell cycle. RB halts progression of the cell cycle and E2F promotes progression through upregulation of cyclin D1 production. This balance of RB proteins binding and inhibiting E2F and CDK4/6-cyclinD phosphorylating and inhibiting RB regulates cell cycle progression. A CDK4/6 inhibitor may resensitize cells to a MEKi. We first addressed this target by utilizing on-target siRNA to inhibit CDK4 in our resistant cell lines. This knockdown leads to a reduction in cell viability when combined with the use of a MEKi. To validate the findings of the role of this pathway mediating MEKi sensitivity we also knocked down RB and found that normally sensitive cell lines could be converted into resistant cell lines.

The use of palbociclib, which is an extremely potent and highly specific inhibitor of CDK4/6, in conjunction with a MEKi in KRAS-mutant NSCLCs, represents a potential treatment option for KRAS-mutated NSCLC patients. This combination of treatment has been shown to be significantly synergistic in melanoma cells. We have shown for the first time in KRAS-mutant NSCLCs that combination MEKi and CDKi can be used in cell lines with mutant p16 to enhance the efficacy of the treatment.

Conflicting interests

The authors have declared that no conflict of interests exist.

References


