Analysis of Resistance Mechanisms in NSCLC Tumor Cells to the Checkpoint Inhibitor Prexasertib

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Abstract: This study analyzes mechanisms of resistance to the checkpoint inhibitor Prexasertib in squamous lung adenocarcinoma cells (NCI-H520). Using long-term exposure to incremental doses of Prexasertib, researchers identified shifts in cell signaling, particularly in pathways related to cell cycle regulation, DNA repair, and apoptosis evasion. Our findings indicate MAPK and E-Cadherin pathway's activation as major factors in sustaining cell proliferation despite treatment, suggesting its potential as a target to overcome drug resistance.

Introduction

Prexasertib, a checkpoint inhibitor (CHK1,2) developed by Eli Lilly, targets the inhibition of CHEK1 and to a limited percentage inhibition of CHEK2 proteins. Its mechanism of action involves the suppression of CHK1,2, which leads to a stabilization of Cdc25A. This accumulation of Cdc25A leads to activation of CDK2 even in the presence of DNA damage. This bypass of the intra-S checkpoint results in DNA fragmentation, replication catastrophe, and cell death. This study investigates the specific mechanisms of Prexasertib induced resistance in squamous lung adenocarcinoma cells (NCI-H520) and explores the underlying molecular pathways.

Methods

A long-term experiment with constant minimal dosing of Prexasertib, beginning at 2 nM and gradually reaching a final concentration of 75 nM over 60 days, was used on NSCLC cells (NCI H520) to generate resistant cell lines [1]. This study compared untreated parental NCI H520 cells with Prexasertib-resistant NCI H520 cells to identify differences in cell signaling and uncover potential markers and pathways involved in resistance development. Using Hyperplex™, a 1500-plex cell signaling assay, the study measured a total of 300 protein markers, including phosphoproteins, to quantify changes in protein expression and the activation of 60 distinct signaling pathways.

Results

The analysis revealed a multitude of regulated signaling proteins between parental and resistant cells (Fig. 1). Significant changes were observed in cell cycle regulation, DNA damage repair, epithelial-mesenchymal transition (EMT) involving key players like E-cadherin, immune signaling, stem cell signaling, and apoptosis.

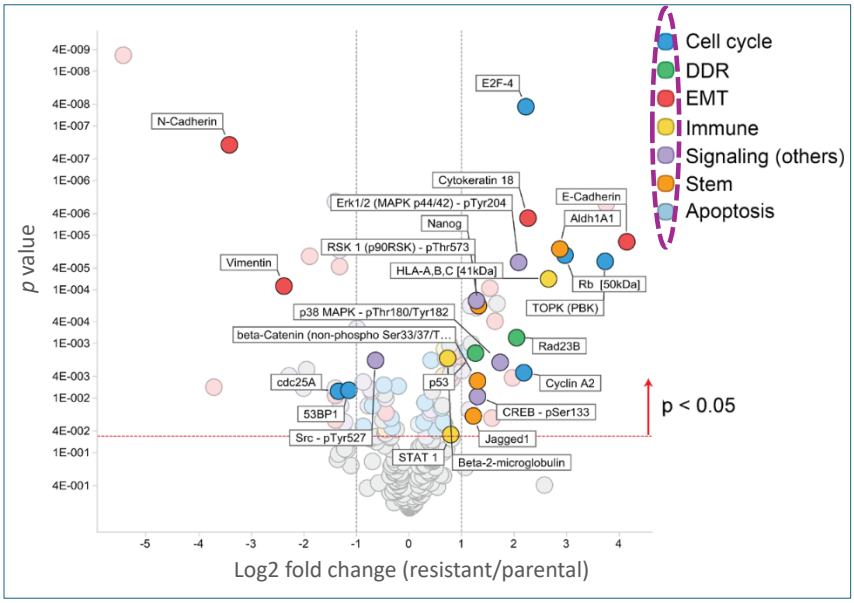


Fig. 1: Vulcano plot of Hyperplex™ assay results. Significant regulation (p<0,05 and log2 fold) between parental and resistant cell lines was found in cell signaling pathways correlating to cell cycle, DNA-Damage Repair (DDR), Epithelial-Mesenchymal Transition (EMT), Immune-, Stemcell- Apoptosis and other not further specified signaling pathways

Cancer Resistance Analysis

The data was visualized through pathway maps, facilitating the validation of correlations and insights into causal relationships (Fig. 2). Two key pathways were notably activated in resistant cells and deemed significant:

1. **E-Cadherin Pathway**: This pathway can evade apoptosis and was found to be upregulated in resistant cells.
2. **MAPK Pathway**: This pathway regulates cellular proliferation cascades and showed increased activity in resistant cells.

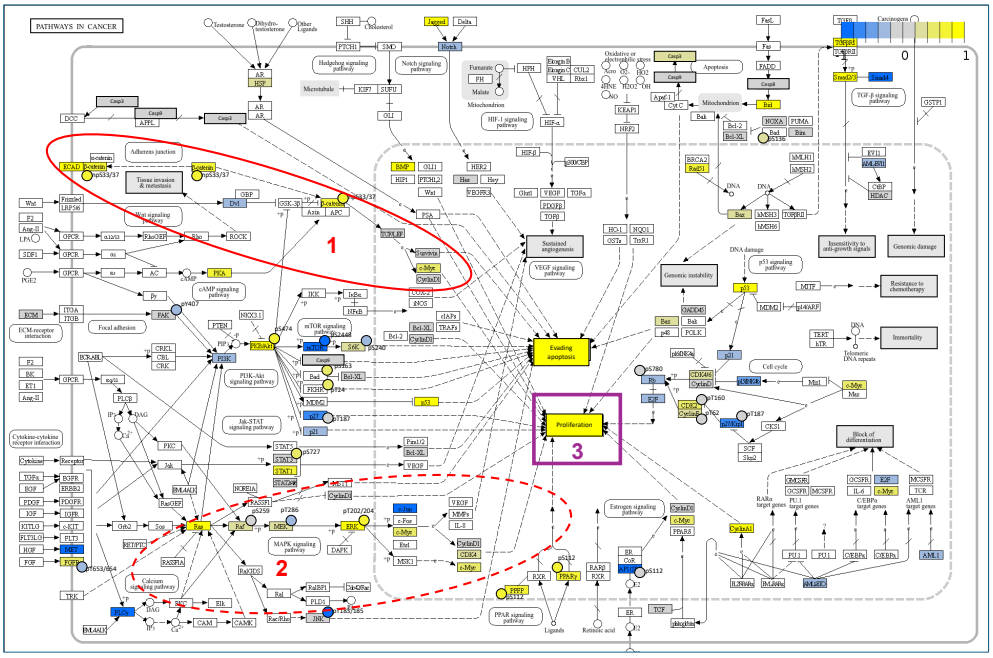


Fig. 2: Visualization of pathways relevant in cancer progression. Significant regulation was found in proteins of the E-cadherin pathway (red circle 1) and the MAPK-Pathway (red dashed circle 2). The E-cadherin pathway leads to upregulation of the signaling pathway connected to evading apoptosis (yellow bar) while the MAPK-Pathway increases activity in the Proliferation pathway (purple square 3).

To better understand Prexasertib´s mechanism of action and the underlying consequences in resistant tumor cells, a detailed depiction of the proliferation signaling pathway (see Fig.2 purple square 3) was conducted. The proteomics data were mapped onto proliferation-related pathways to confirm causal relationships (Fig. 3).

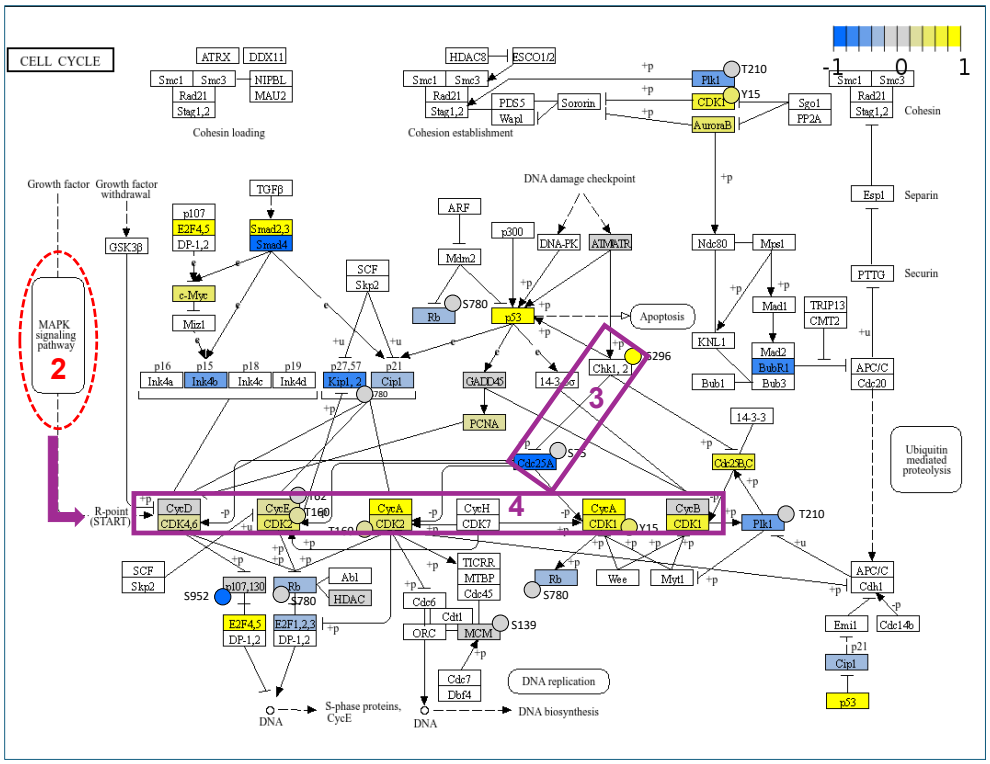


Fig.3: Visualization of pathways relevant for cell proliferation. While the Checkpoint proteins Chk 1, 2 show upregulated phosphorylation, the corresponding effector target Cdc25A is downregulated (purple square 3). In a downregulated state Cdc25A does not have significant impact on the CDK signaling pathway even though the entire cascade is upregulated (purple square 4). The signaling impulses for CDK-upregulation is induced by the upregulated MAPK signaling pathway (red dashed circle 2; fig. 2 red dashed circle 2)

Discussion

Resistant cells exhibited upregulation of phosphorylated CHK1/2, but a significantly lower concentration of CDC25A compared to parental cells. (Fig. 3: purple square 3). Despite the downregulation of CDC25A, the CDK signaling cascade was not downregulated; rather, it was upregulated (Fig. 3: purple square 4). This upregulation supports continued DNA replication and cell proliferation in the resistant cell lines. As a result, despite treatment with Prexasertib, CHK1/2 inhibition in these cells remained susceptible to reductions in efficacy. Additionally, in the resistant cells an alternative pathway is activated—specifically, an upregulated MAPK pathway—responsible for activating the CDK signaling cascade and driving DNA replication and proliferation. (Fig. 3).

The up-regulated activity of the MAPK pathway in resistant cells supports the hypothesis that these cells can evade the regulatory effects of the CHK1,2 inhibitor. Through activation of the MAPK pathway, they not only proliferate without restraint but also at an increased rate. This suggests that the MAPK pathway plays a critical role in the development of resistance to Prexasertib.

Conclusion

The findings indicate that resistance to Prexasertib in NCI-H520 tumor cells is closely associated with altered CDC25A regulation and the activation of alternative pathways, particularly the MAPK pathway. In resistant cells, the reduced expression of CDC25A decreases the efficacy of CHK1/2 inhibition. Concurrently, MAPK pathway activation supports continued cell proliferation despite treatment.

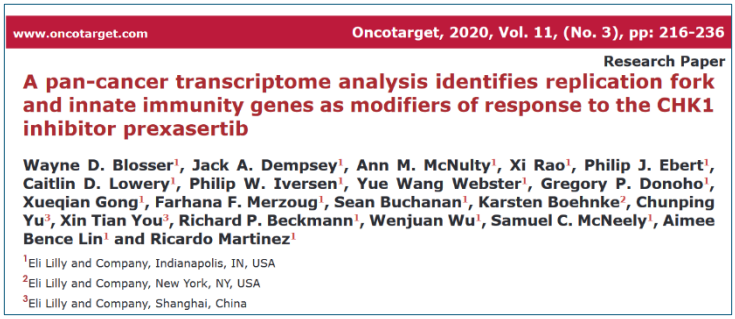
Prexasertib has demonstrated limited therapeutic impact in larger patient populations, partly due to adverse events that restrict dosing flexibility and thus narrow its therapeutic window. As a result, resistance frequently develops in clinical settings where dosing flexibility is constrained.

These results suggest that targeting the MAPK pathway may be an effective strategy to counteract resistance in Prexasertib-treated tumor cells. Further research is necessary to elucidate these resistance mechanisms and to evaluate the potential of combining Prexasertib with other therapeutic strategies.

Note: In April 2019, Eli Lilly discontinued the development of Prexasertib (LY2606368). The company subsequently published Prexasertib signaling data in 2020, and Prexasertib is now available through commercial vendors for research purposes.

Reference

[1] Blosser W. D., Dempsey J. A., McNulty A. M., Rao X., Ebert P. J., Lowery C. D., Iversen P. W., Webster Y. Wang, Donoho G. P., Gong X., Merzoug F. F., Buchanan S., Boehnke K., et al A pan-cancer transcriptome analysis identifies replication fork and innate immunity genes as modifiers of response to the CHK1 inhibitor prexasertib. Oncotarget.; 11: 216-236.



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Research Paper

A pan-cancer transcriptome analysis identifies replication fork and innate immunity genes as modifiers of response to the CHK1 inhibitor prexasertib

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