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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Inhibition of Lysine Acetyltransferases p300/CBP Overcomes BTK Inhibitor Resistance in Mantle Cell Lymphoma Cells

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Background: Although Bruton's Tyrosine Kinase inhibitors (BTKis) are effective against relapsed refractory mantle cell lymphoma (MCL), the limited duration of therapeutic response and the emergence of resistance remain clinical issues. We conducted a CRISPR/Cas9 screen to identify the genes responsible for BTKi sensitivity and resistance.
Materials and Methods: To identify the genes responsible for BTKi resistance, we used a CRISPR library (Kosuke Yusa, V3), targeting 18,740 human genes. We compared three BTKis, ibrutinib, acalabrutinib and tirabrutinib, using DMSO as a control. We transduced Granta519, an ibrutinib-resistant MCL cell line expressing Cas9 with the CRISPR library lentiviruses. Next, we counted sgRNA copy numbers using next generation sequencing after a two-week drug treatment. We identified 76 genes that sensitized cells to the BTKis upon knockout. After excluding common essential genes based on the Cancer Dependency Map portal (depmap.org/portal/), we ranked these genes according to the extent to which the combination of their knockout and BTKis inhibited cell proliferation.
Results: Lysine acetyltransferases (KATs), p300 and CBP were ranked first and ninth, respectively. Knockout of p300 or CBP alone moderately decreased cell numbers, where as their combination with BTKi treatment significantly decreased cell numbers.
To further validate this synergistic effect, we performed an MTS assay in combination with p300/CBP inhibitors (C-646 and A-485) and ibrutinib in MCL cell lines (Granta519 and Mino). The p300/CBP inhibitor increased ibrutinib sensitivity, leading to a up to four-fold greater suppression of cell viability. This effect was even greater than that of existing drugs, including venetoclax (a BCL-2 inhibitor), and idelalisib (a PI3K inhibitor), which are thought to have synergistic effects. The synergistic effect of a p300/CBP inhibitor and ibrutinib was also observed in long-term cultures for up to two weeks in both ibrutinib-resistant and ibrutinib-sensitive MCL cell lines. Furthermore, combination treatment resulted in an enhancement of G0/G1 cell cycle arrest and an increase in apoptotic cells in the MCL cell lines.
To elucidate the mechanism underlying this synergistic effect, we measured the expression of IκBα mRNA as an indicator of NFκB activity by real-time PCR. In Granta519, ibrutinib alone caused an initial decrease in IκBα mRNA expression followed by a rebound increase. However, the addition of A-485 decreased the rebound and suppressed IκBα mRNA expression. These results indicate that ibrutinib and A-485 cooperatively suppress NFκB activity.
The combination of ibrutinib and A-485 synergistically suppressed phosphorylation of PLCγ2 and of AKT as confirmed by Western blotting. This suggests that downstream PLCγ2 and AKT activation might be involved in ibrutinib resistance. Lentiviral overexpression of p300 in Granta519 cells enhanced phosphorylation of AKT. On the other hand, ibrutinib suppressed the expression of p300, and conversely, p300/CBP inhibitors suppressed the expression of BTK. These data suggest that the expression of p300 and BTK are dependent on each other. We hypothesize that direct or indirect interactions may occur through phosphorylation by BTK and acetylation by p300.
Conclusion: Our CRISPR screen identified lysine acetyltransferases p300/CBP as BTK inhibitor resistance genes in human MCL cells. The combination of a p300/CBP inhibitor and a BTK inhibitor synergistically suppresses both BTK and AKT, leading to NFκB suppression. Taken together, we first identified that the combination of p300/CBP inhibition and BTK inhibition could be a promising strategy for treating patients with MCL.

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