Chk1 inhibition and Wee1 inhibition combine synergistically to inhibit cellular proliferation

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Introduction

Inhibition of the checkpoint kinase Chk1, both as a monotherapy and in combination with DNA damaging cytotoxics, is a promising therapeutic strategy for cancer. However, most remains to be learned in regard to the patient populations that will respond best to a Chk1 inhibitor and the optimal therapeutic to combine with a Chk1 inhibitor. To explore this inter-grouping of combinations and novel combination strategies for Chk1 inhibition, we performed a ‘synthetic lethality’ screen with the selective Chk1 inhibitor Chk1-A. This screen employed a custom made library of siRNAs against 197 genes (3 siRNAs per gene), most of which are involved in cell-cycle control or DNA damage repair. One of the most prominent and consistent hits across runs of the screen performed in PC3, LNCaP, and A549 cell lines was Wee1 kinase. MK-1775 is a small molecule inhibitor of Wee1 that is currently in early stage clinical trials. In confirmation of the results obtained from the siRNA screen, we found that Chk1-A and MK-1775 synergistically inhibited proliferation in multiple cell lines. This anti-proliferative synergy correlated with a synergistic induction of apoptosis. We explored the mechanism of the impressive synergy by examining the cellular and biochemical effects of the Chk1-A and MK-1775 combination. We found that treatment of the two inhibitors resulted in dramatic decrease in inhibitory phosphorylation of cyclin-dependent kinases 1 and 2, increases in DNA damage, and the collapse of DNA replication. In conclusion, the combination of a Chk1 inhibitor and a Wee1 inhibitor may be an effective treatment strategy for cancer.

Results

Figure 2: Wee1 a prominent hit in synthetic lethality screens

Figure 3: Chk1-A and MK-1775 synergistically inhibit proliferation in multiple cell lines

Figure 5: Chk1-A and MK-1775 have differential effects on the cell-cycle, premature mitosis is cell-type dependent

Figure 6: Chk1-A and MK-1775 both induce CDK inhibitory phosphorylation and increase H2A.X phosphorylation, but only Chk1-A leads to strong cell-cycle checkpoint activation

Figure 9: Chk1-A and MK-1775 have differential effects on progression through S-phase in HEL92.1.7 cells

Figure 1: Description of the synthetic lethality siRNA screen

Figure 4: Chk1-A and MK-1775 synergistically induce apoptosis in HEL92.1.7 cells

Figure 8: Chk1-A treatment results in collapse of DNA synthesis in S-phase and the addition of MK-1775 enhances this effect

Figure 7: Chk1-A but not MK-1775 induces loading of Cdc45 onto DNA

Conclusions

- Chk1A inhibition and Wee1 inhibition synergistically inhibit proliferation and induce apoptosis
- Chk1A inhibition and Wee1 inhibition both lead to reductions in inhibitory phosphorylation of CDKs and an increase in H2A.X phosphorylation, and the combination of the inhibitors enhances these effects
- Chk1A inhibition, but not Wee1 inhibition, leads to excessive loading of Cdc45 onto DNA and collapse of DNA replication early in S phase
- Wee1 inhibition, but not Chk1A inhibition, leads to early histone H3 phosphorylation and accumulation in S phase
- Synergistic inhibition of proliferation may be due to the combination of these differential effects on the cell cycle