Abstract

Introduction:
This study describes the development of a novel dual specificity kinase inhibitor, ON123300, which exhibits potent activity against colorectal cancer both in vitro and in vivo. Over-expression of Cyclin D1 has been found to closely correlate with the proliferation rate of the tumor cells, metastatic colorectal cancer over expresses ARK5, a member of the AMPK family which has been shown to mediate Akt activation. In this study, we show that ON123300, which blocks CDK6 and ARK5, is a potent inducer of apoptosis of colorectal cancer cells when combined with palbociclib, a highly selective inhibitor of CDK4/6 that does not target ARK5.

Results and Conclusions:
We examined the effects of palbociclib and ON123300 on cell proliferation, the regulation of the PI3K/AKT, and induction of apoptosis in multiple colorectal cancer cell lines. Comparative kinase inhibition assays showed that while palbociclib and ON123300 exhibited comparable inhibitory activity against CDK4/6, ARK5 activity was inhibited only by ON123300. When DLD1 and SW480 cells were incubated with ON123300, similar concentrations of palbociclib, both compounds were equally efficient in their ability to inhibit phosphorylation of three members of the Rb family of proteins. However, when the phosphorylation status of proteins associated with the PI3K/AKT pathway was measured by western blot analysis, ON123300 showed concentration-dependent inhibition of 4EBP and S6K phosphorylation while palbociclib had no or little effect on the phosphorylation of these proteins. Cells treated with palbociclib rapidly accumulated in the G0/1 stage of the cell cycle with increased drug concentrations. Although cells treated with ON123300 also arrested in the G0/G1 phase at lower concentrations (0.1–0.5 µM), with increasing concentrations of drug, there was an accumulation of cells with sub-G1 DNA content, suggesting induction of apoptosis. ON123300-treated cells showed cleavage of PARP and CASPASES (3,7,9) as well as inhibition of FOX01 activity. Treatment of SW480 colorectal cancer cells with ON123300 resulted in an increase in glucose uptake, profound inhibition of glutamine uptake and reduced ATP production.

Inhibition of PI3K-mediated Signaling by ON123300.

CONCLUSION

1. Palbociclib and ON123300 exhibited equivalent potency in the inhibition of CDK4/6 kinase activity whereas ARK5 activity was inhibited only by ON123300.

2. Palbociclib and ON123300 were equally efficient in their ability to induce phosphorylation of all three members of the Rb family of proteins.

3. ON123300 showed concentration-dependent inhibition of 4EBP and S6K phosphorylation while palbociclib had no or little effect on the phosphorylation of these proteins.

4. A dual inhibition strategy mediated significant alterations in the levels of metabolites associated with glutamine metabolism.

5. DLD1 and SW480 were selected for a xenograft model, suppression of ARK5 and CDK4 pathway could be an effective therapeutic strategy for the treatment of colorectal cancer.

REFERENCES


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