The Mechanism of Mycophenolic Acid Cytotoxicity

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Abstract

Mycophenolic acid (MPA), a well-known inhibitor of de novo purine biosynthesis, blocks the enzyme inosine monophosphate dehydrogenase (IMPDH) and in doing so blocks the synthesis of guanosine monophosphate (GMP). MPA has previously been tested in mice and in human patients. MPA has been shown to decrease cancer cell migration *in vitro* as well as promote apoptosis and decrease tumor burden in mice. In order to evaluate the specificity of MPA in pancreatic cancer cells, we tested the inhibitor against a panel of pancreatic cancer cell lines, namely, CFPAC1, S2-013, and KPC cells. We evaluated the dose response (IC50) of CFPAC1, S2-013, and KPC cells to MPA under both normoxia and hypoxia, and then investigated the potential for rescuing the cell viability with various metabolites. Hypoxia had modest impact on MPA responsiveness of pancreatic cancer cell lines. We observed rescue of cell viability upon MPA treatment by using guanine, guanosine, and 2'-deoxyguanosine; however, the viability could not be effectively rescued by hypoxanthine or xanthine. We also performed mass spectrometry-based metabolomic analysis to investigate the levels of metabolic alterations in response to MPA treatment. As expected, there was a significant increase in the concentration of inosine and a decrease in the concentrations of xanthine monophosphate (XMP) and GMP with no significant effect on the concentration of adenosine or argininosuccinic acid. Unexpectedly, we noted a significant increase in the concentration of hypoxanthine upon MPA treatment. These results suggest that MPA may block the degradation or salvage of hypoxanthine. Further studies will be needed to confirm and determine the mechanism of hypoxanthine accumulation in MPA-treated cells.

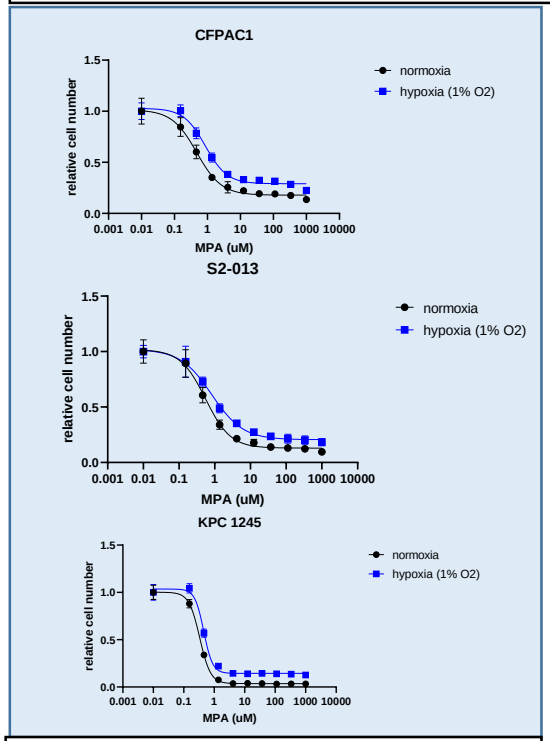


Figure 1. Cells were cultured under normoxia (37°C, 5% CO_2) or hypoxia (37°C, 1% O_2 5% CO_2) with varying concentrations of MPA. Cells were counted using Hoechst stain and normalized to untreated controls.

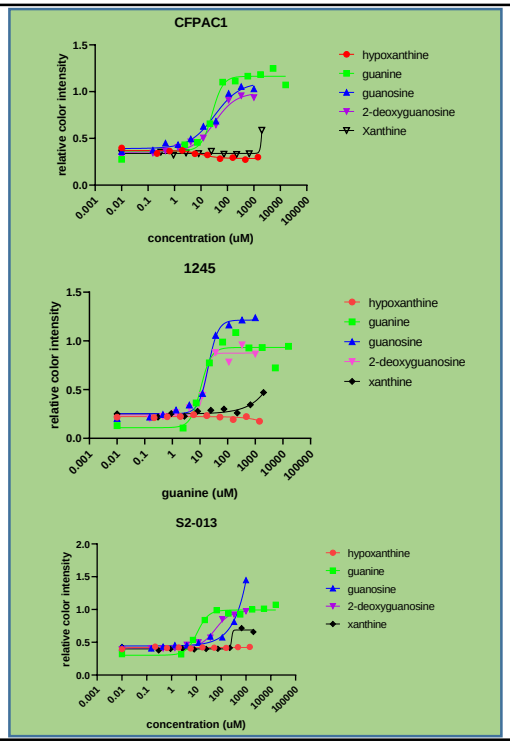
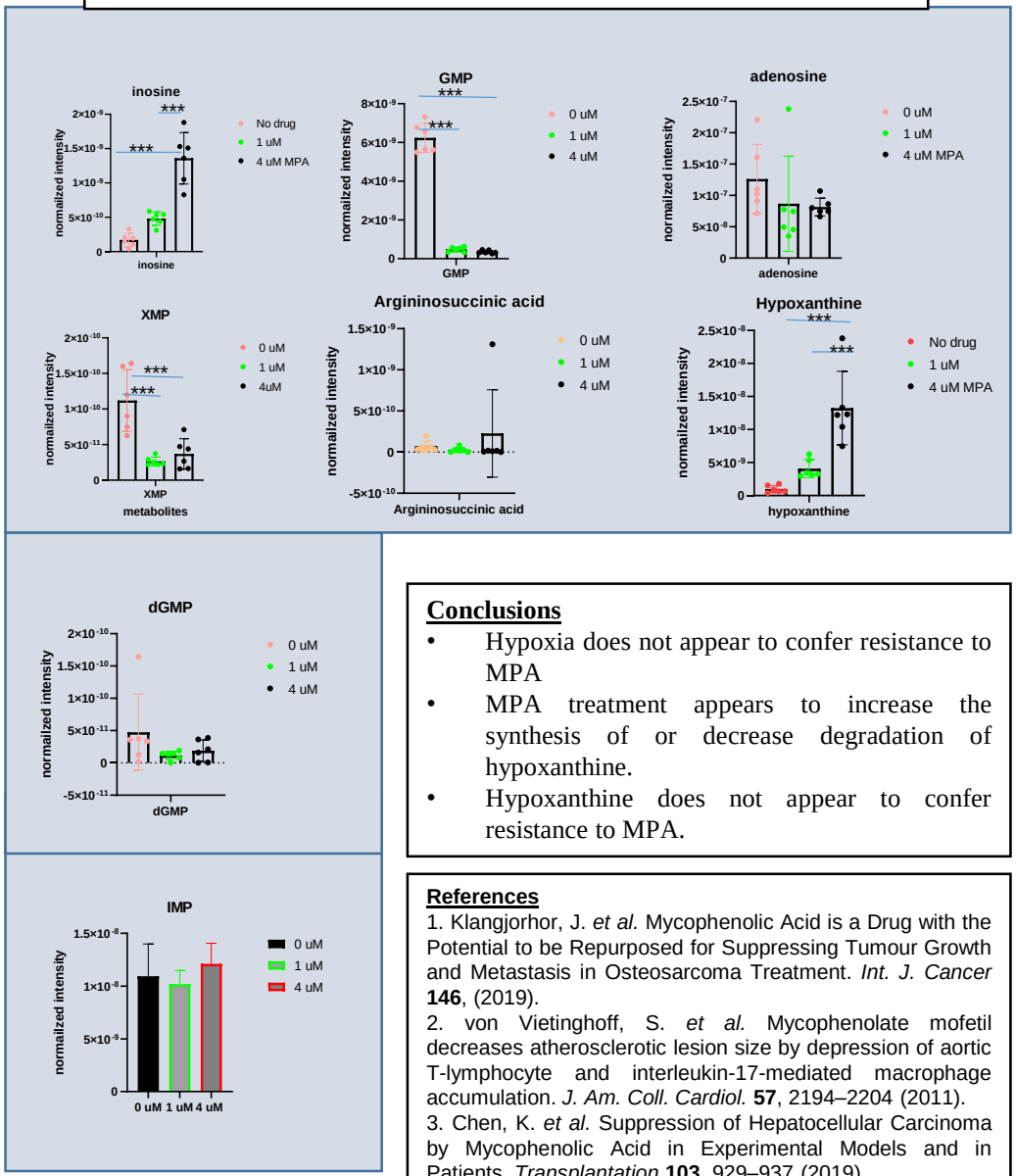


Figure 2. Rescue from MPA with various metabolites. Given as a ratio of cells treated with MPA (12 μM for CFPAC1 & 4 μM for S2-013 & KPC1245). Cell viability was measured via MTT assay and normalized to untreated controls..

Figure 3. Mass-spec measurements of metabolites in S2-013 cells treated with MPA. ***=p<0.001, **=p<0.01, *=p<0.05



Conclusions

- Hypoxia does not appear to confer resistance to MPA
- MPA treatment appears to increase the synthesis of or decrease degradation of hypoxanthine.
- Hypoxanthine does not appear to confer resistance to MPA.

References

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2. von Vietinghoff, S. *et al.* Mycophenolate mofetil decreases atherosclerotic lesion size by depression of aortic T-lymphocyte and interleukin-17-mediated macrophage accumulation. *J. Am. Coll. Cardiol.* **57**, 2194–2204 (2011).
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