Saquinavir Increases Phosphorylated Eukaryotic Elongation Factor 2 (peEF2) at the Anal Transition Zone in Transgenic Mice

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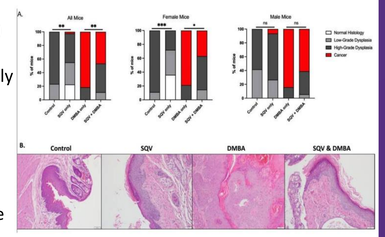
Introduction

- Anal cancer has seen its rates rising steadily for decades, with significant increase noted from the early 2000's to the present
- There were more than 10,000 new documented cases of anal cancer in 2022 alone, with over 1,600 deaths attributable to the disease
- Human Papilloma Virus (HPV) is believed to be a contributing factor in anal cancer development in over 90% of documented cases
- Recent CDC data estimates that roughly 75% of Americans have received at least one vaccination against HPV
- The current treatment guidelines for anal cancer follow the Nigro Protocol, first established in 1974
- Although current treatments are effective, the lack of preventative options is the ultimate driving force behind this study

Background

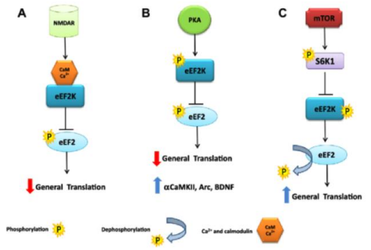
- Saquinavir (SQV) is an FDA-approved Protease Inhibitors (PIs) for the treatment of HIV
  - Previously shown to significantly reduce cancer development in HPV infected mice
  - Saquinavir inhibits cell proliferation through the depletion of E6 and E7 oncoproteins, which contribute to the carcinogenesis of HPV
  - This study was concerned with exploring other modes of anti-proliferation potentiated by SQV



Gunder et al., 2023

Study Question

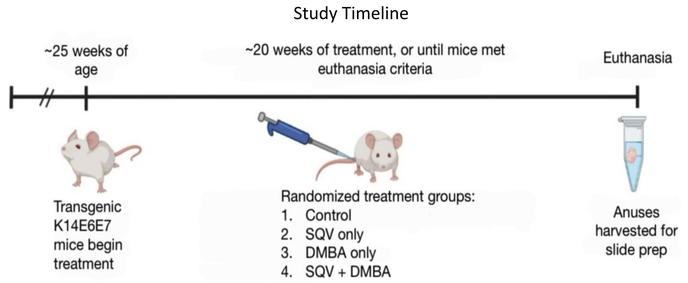
- Eukaryotic Elongation Factor 2 (eEF2) has previously been shown to be elevated in multiple cancers
- peEF2, the inactivated state of eEF2, can be used to quantify the suppression of polypeptide elongation
- We hypothesized that mice treated with SQV would show increased levels of localized peEF2 staining at their anal transition zones (ATZ)



Taha et al., 2013

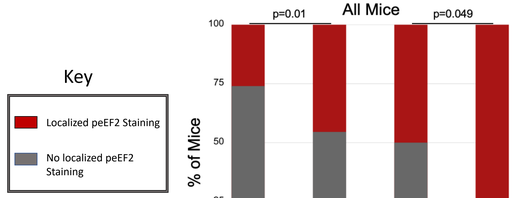
Methods

- K14E6/E7 mice, which are bred with continually active E6 and E7 oncoproteins, were used in this study
- Utilizing K14E6/E7 mice enables us to reproduce high grade anal dysplasia and subsequent tumor growth
- At 25 weeks of age, when high grade anal dysplasia was established, mice were randomly selected to treatment groups
- Mice received weekly treatments of SQV, DMBA (a carcinogen), SQV + DMBA, or control until completing 20 weeks of treatment or euthanasia criteria were met



Results

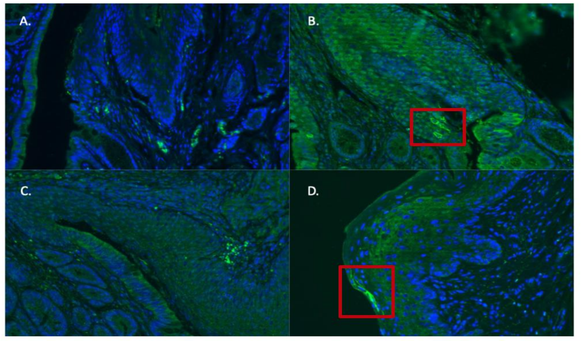
- Mice treated with SQV showed significantly increased levels of phosphorylated eEF2 staining when compared to their respective controls
- The group of mice treated with SQV only showed significantly more mice with localized peEF2 staining at the ATZ when compared to control.
- The group given DMBA + SQV showed significantly more mice with localized peEF2 staining at the ATZ when compared to mice that received DMBA only.



Imaging

A. Control
B. SQV only
C. DMBA only
D. SQV + DMBA

- These images illustrate the transition from columnar epithelium to stratified squamous epithelium at the anal transition zone in treated mice
- The images from mice treated with Saquinavir have been highlighted to display localized areas of peEF2 staining
- Images were analyzed with FIJI to quantify areas of heightened peEF2 staining



Conclusion

- Treatment groups that received SQV showed significantly more mice with localized areas of peEF2 staining
  - This suggests that Saquinavir works to inhibit cell proliferation in HPV infected mice through the inactivation of Eukaryotic Elongation Factor 2
  - This finding reinforces the potential of Saquinavir as an anti-tumorigenic agent, warranting further study

Future Plans

- Saquinavir will be used in trials with eEF2 knockout mice to determine if inhibiting this pathway is necessary for anti-tumorigenesis in mice infected with papilloma virus
- Phase 1 clinical trials have recently been initiated at UW-Health to determine the parameters of Saquinavir as an anal cancer preventative

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