Introduction
Pancreatic Cancer is one of the deadliest cancers with 6 months being the median patient survival with current available therapies. My current laboratory has shown that inhibition of Eukaryotic Elongation Factor 2 kinase (EF2K) by siRNA technology suppresses cell proliferation of various cancer cells. EF2K is an alpha kinase which phosphorylates eukaryotic elongation factor 2 to create p-EF2, inhibits EF2 function, regulates protein translation and is heavily involved in cell division and cell proliferation. However, there was no clinically applicable inhibitors of EF2K till Thymoquinone (TQ). TQ is a compound found in Nigella Sativa that has anti-cancer, pain relieving and anti-inflammatory and many other capabilities. Thus, Thymoquinone is thought to be a possible agent in fighting cancer. However, the mechanism by which TQ inhibits pancreatic cell proliferation has yet to be established. We hypothesize that TQ suppresses Pancreatic Cancer cell proliferation through inhibition of EF2K activity. By inhibiting EF2K activity, a dose dependent downregulation of p-EF2 should be seen.

Methodology
To prove the hypothesis, several clonogenic assay and Western Blots were set up in order to review the role of Thymoquinone in inhibiting EF2K.

Western Blot
Four T-25 flasks were seeded with 10,000 Panc-1 cells, labelled with the concentrations of thymoquinone they were to be treated with and left to proliferate and attach to the flask for 48 hours. After 48 hours, treatments of Thymoquinone were added to the flasks. The concentrations were as follows 2.5uM, 5uM and 10uM with control, DMSO. After the treatment was added, the cells were left for another 48 hours. After 48 hours, the cells were collected, lysed, quantified and run. After the run, the membrane was transferred and the primary antibody, p-EF2 was applied to measure the down regulation of EF2K activity.

Clonogenic Assay
In a 6-well plate, 1000 Panc-1 cells were seeded in 2ml of media. These cells were left for 48 hours before being treated with Thymoquinone of concentrations, 2.5uM, 5uM, 7.5uM and 10uM with 2 additional controls, one well left untreated and one well of 1ml of DMSO. 9 days after treatment, the cells were stained and checked for decrease in cell proliferation.

Results

Clonogenic Assay
After staining each well of the plate, it was seen that with increasing concentration of Thymoquinone, colony formation decreased with the IC50 being 5uM of Thymoquinone. This experiment also proves that Thymoquinone inhibits cell proliferation in Pancreatic Cancer. These results can be seen Figure 1.

![Figure 1: Duplicate Clonogenic Assay done using Panc-1 cells and increasing concentration of Thymoquinone](image)

Western Blot
On reading the membrane gotten from the gel electrophoresis, the hypothesis was seen to be true. With increased concentration of Thymoquinone, the expression of p-EF2, a marker to test whether EF2K activity had reduced, was seen to decrease. This can be seen in Figure 2. This shows that Thymoquinone could possibly serve as novel drug in combatting pancreatic cancer by decreasing its proliferation by EF2K. Figure 3 shows that Thymoquinone inhibits EF2K activity and not EF2K directly. This is shown by the constant band of EF2K along the increasing Thymoquinone concentration.

![Figure 2: Down Regulation of p-EF2 with increasing Concentration of Thymoquinone](image)

![Figure 3: Western Blot of EF2K. Constant amount of EF2K with increasing concentration of Thymoquinone](image)

Discussion/Conclusion
As discussed previously, the hypothesis of this experiment was that Thymoquinone can inhibit the proliferation of Pancreatic Cancer cells by inhibiting EF2K activity. To prove this hypothesis, Clonogenic assays and Western Blots analysis were performed. The western blot proves the inhibition of proliferation by the EF2K pathway by showing the reduction in the expression of p-EF2 with increasing concentration of Thymoquinone. Additionally, the clonogenic assay proves the hypothesis by showing that with increasing concentration of Thymoquinone, there is a decrease in colony formation of the cells. This show that TQ dose-dependently inhibits EF2K activity in Pancreatic Cancer cells. These results coincide with previous results that show that the inhibition of EF2K gene by siRNA reduced pancreatic cell proliferation. In conclusion, our studies suggest that TQ inhibits pancreatic cancer cell proliferation through EF2K activity inhibition. These findings contribute to cancer research by proposing an alternative drug to treating Pancreatic Cancer. Though, there are many more steps to go before Thymoquinone can finally be used as a drug in fighting Pancreatic Cancer but a few steps closer in making cancer history.

References
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