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Identifying New Targets In Colon Cancer By Gene Expression Following ERK Kinase Inhibition

A mutation in the MAPK cell signaling pathway is detected in over one-third of all cancers. Most often, it causes hyperactivation of the pathway leading to uncontrolled cell growth. Protein Kinases in the pathway regulate protein activity by phosphorylation, inducing a conformational change in the protein switching from an inactive to active state. In the MAPK pathway, the protein kinases MEK and ERK have critical roles in regulating the cell cycle, phosphorylating transcription factors and initiating negative feedback loops. SCH772984(SCH) and Trametinib (GSK1120212) are two known inhibitors for ERK and MEK, respectively. This research project was designed to explore the changes in gene expression levels in cancer cells after exposure to inhibitors. The three main goals of the research were to confirm literature IC50 values for the compounds, confirm functional target engagement, and identify new genes that were upregulated following inhibition. To conduct the project, Cell Proliferation Inhibition Assays were completed using 96-well plates to measure the number of cells present after incubation with compounds in varying concentrations. The results of the project confirmed an IC50 value of 1.10 nM for Trametinib and 33 nM for SCH. Western Blotting was used to determine the phosphorylation levels of MEK, ERK, and its substrates after drug treatment. To further confirm target protein inhibition, RNA from treated cells was extracted, purified, and converted to cDNA. Expression levels of selected genes known to decrease following treatment were determined by amplification using an RT-PCR assay. For example, the gene expression levels of DUSP4 (phosphatase) and MYC (transcription factor) decreased following ERK inhibition. Finally, the expression profile of several selected proteins was analyzed for its increase or decrease of expression in cancers following drug treatment. These results are important as they can help identify new target proteins for inhibition with combination therapy to decrease resistance in different cancers.

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Identification of New Targets in Colon Cancer Cells following ERK Inhibition.

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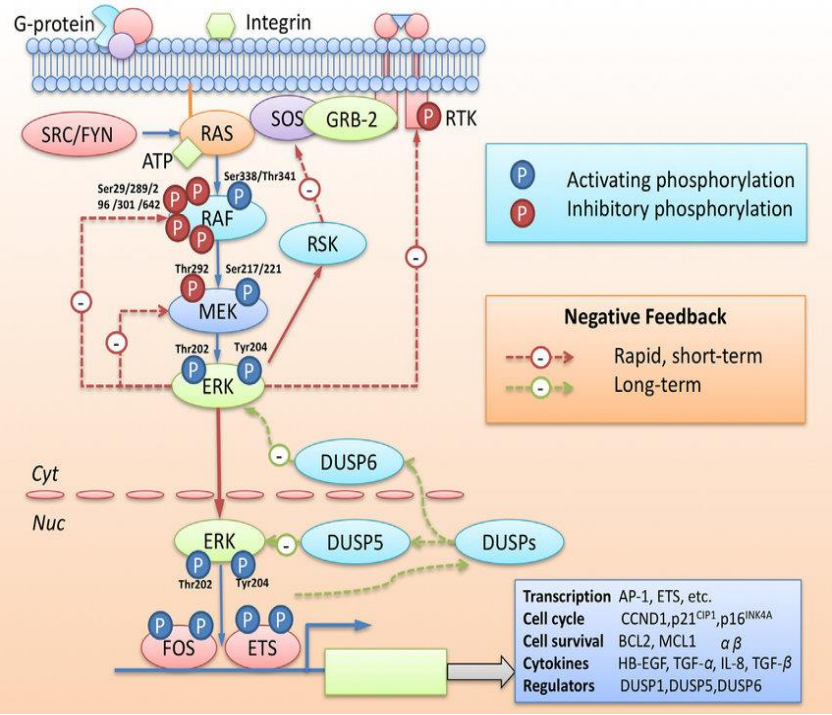
Introduction

A hyperactive MAPK cell signaling pathway (Fig1) is present in over one-third of all cancers due to activating mutations in kinases, KRAS and RAF. Protein kinases MEK and ERK follow KRAS & RAF and hold critical roles in activating gene transcription and uncontrolled cell proliferation through phosphorylation of key signaling proteins. MEK and ERK kinases regulate the cell cycle, phosphorylate transcription factors, and initiate negative feedback loops. SCH772984 (SCH) and Trametinib (TRA) are two known inhibitors for ERK and MEK, respectively. Overtime, patients will develop resistance to these inhibitors. Therefore, it is important to analyze the genes that are being upregulated and downregulated following exposure to compounds to discover potential new targets for cancer treatment using combination therapy.

Figure 1: Overview of the MAPK Signal Transduction Pathway[1]



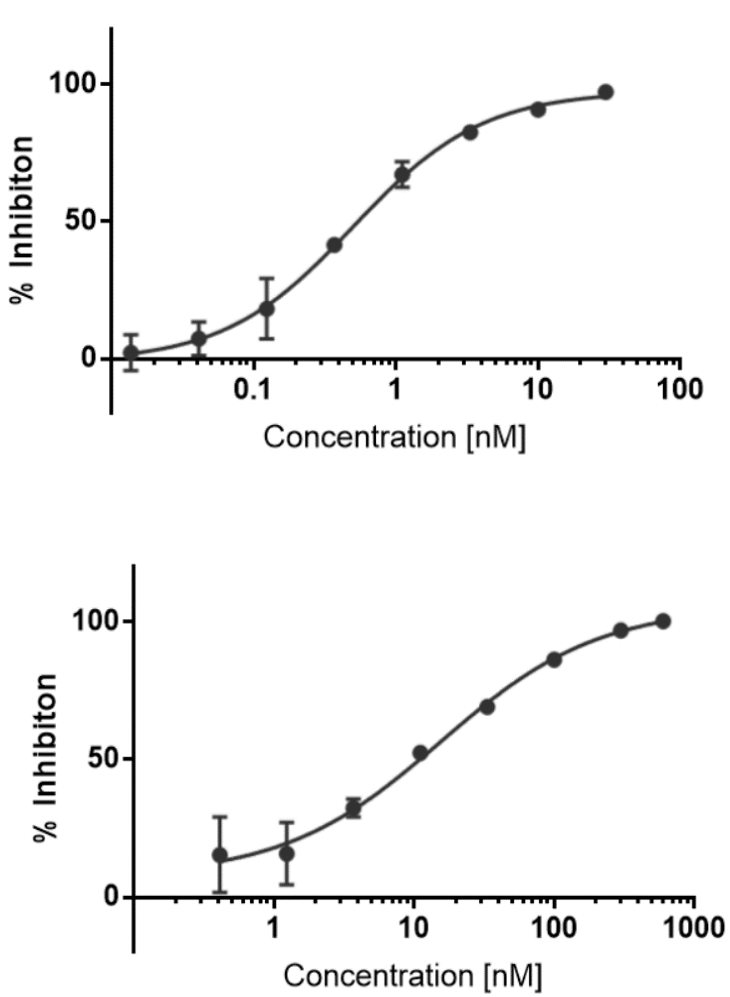
Research Objective: In 2018, Basken et al., used Mass Spectrometry to analyze hundreds of proteins affected by SCH and TRA treatment. The proteins in Table 1 showed a significant fold increase in phosphorylation. Several of these genes were selected to measure their expression level to see if another pathway is being hyperactivated upon MEK and ERK inhibition.

Table 1: Mass Spectrometry Analysis of Phosphorylated Proteins[2]

				SCH	SCH
	Protein names	Gene names	Pi Position in protein	Fold Increase	P Value
1	Nuclear pore complex protein Nup214	NUP214	430	4.90	8.5E-09
2	Ral guanine nucleotide dissociation stimulator-like 3	RGL3	569	3.36	9.1E-05
3	Nuclear receptor corepressor 2	NCOR2	121	2.77	4.0E-05
4	Bcl2-associated agonist of cell death	BAD	134	2.39	1.4E-07
5	Serine/threonine-protein kinase ULK1	ULK1	556	2.37	6.6E-04
6	DNA-directed RNA polymerase I subunit RPA43	TWISTNB	328	2.36	4.5E-05
7	182 kDa tankyrase-1-binding protein	TNKS1BP1	429	2.30	4.1E-04
8	Ataxin-1-like	ATXN1L	330	2.16	6.4E-07
9	DNA-directed RNA polymerase I subunit RPA43	TWISTNB	322	2.10	1.1E-04
10	Nuclear receptor corepressor 2	NCOR2	1444	2.08	4.7E-05

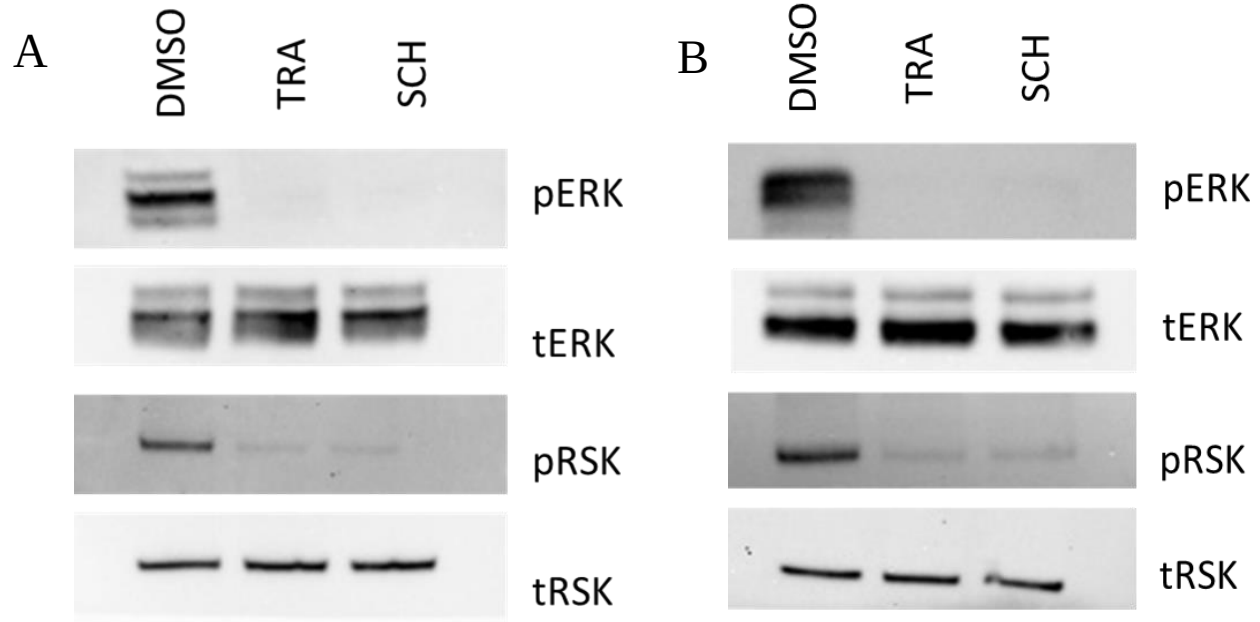
Results Conclusions

Figure 3: IC50 Values of TRA and SCH Compounds



TRA showed an IC_{50} value of 0.51 nM and the SCH had an IC_{50} of 15 nM. The cells were exposed to the compounds at various times points and their phosphorylation levels were detected. Phosphorylation of ERK (pERK) is indicative of activation. The control cells were exposed to 0.1 % DMSO only and a strong pERK band appeared for both time points as expected. After 2 and 8 hours of 2 μM SCH and TRA there was no detectable presence of pERK, demonstrating good inhibition from both compounds. Similarly, there was a very faint band detected at pRSK in both time points, confirming successful target engagement.

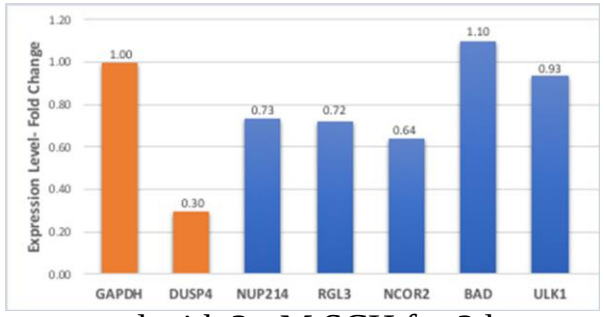
Figure 4: Western Blot of Drug Treated Cells After 2 (A) and 8 (B) Hour Exposure with 2 μM SCH and TRA



Results and Conclusions

Table 2: RT-PCR Gene Expression Change After Treatment

			SCH	SCH	
Protein Names	Gene Names	Pi Position in Protein	Fold Pi Increase	Gene Expression (Fold Δ)	Function
Nuclear pore complex protein Nup214	NUP214	430	4.9	0.73	Nuclear Pore Transport
Ral guanine nucleotide dissociation stimulator-like 3	RGL3	569	3.4	0.72	GTP exchange factor fpor RAL-GTP
Nuclear receptor corepressor 2	NCOR2	121	2.8	0.64	Transcription Regulator
Bcl2-associated agonist of cell death	BAD	134	2.4	1.10	Cell survival
Serine/threonine-protein kinase ULK1	ULK1	556	2.4	0.93	Kinase regulating Autophagy
Controls					
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH			1.00	Expression Control
Dual specificity protein phosphatase 4	DUSP4			0.30	Down Regulated Control



HT-29 Colon cancer cells were treated with 2 μM SCH for 2 hours and the expression level of certain genes was measured using RT-PCR. GAPDH was used as the control in the experiment and successfully showed no change in expression.DUSP4 was used as the positive control and showed an expected decrease in gene expression. The genes in Table 2 were selected from Table 1 and genes NUP214, RGL3 and NCOR2 showed only a moderate decrease in expression (~ 0.6-0.7), indicating that the activation of kinases in another pathway may have increased the phosphorylation of these 5 proteins.

Future Plans:

The results of the project confirm literature IC_{50} values as well as inhibition of pERK and pRSK. A decrease in gene expression for control target DUSP4 indicates inhibition of the pathway. The slight change in gene expression in the 5 selected genes suggests increased phosphorylation is not due to expression increases. These phosphorylation increases may be due to the activation of other kinases following ERK & MEK inhibition.

Future plans include:

1.) evaluation of the remaining 15 genes and

2.) inhibition of enzymes like ULK1 from Table 1 to identify combinations that would synergize with SCH & TRA to inhibit cell proliferation and prevent resistance from developing in cancer cells.