KRAS.G12C Mutation and Effects on Proliferation in Human Lung Cancer

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Abstract

KRAS is a small GTPase whose normal functions are involved in cell signaling, growth, maturation, and death. A point mutation in *KRAS* is a known genetic driver of lung cancer, particularly related to tobacco use. The purpose of the current research is to transform bacterial cell with a plasmid containing mutated *KRAS* and a hygromycin resistance cassette that can be isolated and introduced into human lung cancer lines H1299 and A549. The goal is to produce stable expression of the exogenous mutant KRAS to allow for further analysis of sensitivity to gene therapy and chemotherapy treatments. Polymerase chain reaction (PCR) processes were used to amplify *KRAS* for cloning into a hygromycin resistance cassette containing plasmid using restriction enzymes NheI and NotI. T4 DNA Ligase was used to construct *KRAS*/Hygro plasmids, and the resultant construct was transformed into bacterial cells for storage and retrieval. The goal is to transfect construct plasmids into human lung cancer cell lines A549 and H1299.

Introduction

KRAS is a member of the *Ras* gene family which all encode G proteins that contain intrinsic GTPase activity contributing to major cell functions including differentiation, proliferations, and apoptosis (Jia et al., 2017). Point mutations within codons 12, 13, or 61 are known to be cancer driver mutations for non-small cell lung cancers. Impaired GTPase activity in mutated G proteins results in constitutive activation of *RAS* signaling within the cell (Luo, 2013). *KRAS* G12C has been linked to lung cancers derived from tobacco use, however *KRAS* mutations in lung adenocarcinomas account for 90% of overall RAS mutations (Luo, 2013; Riely et al., 2009). The background levels of *KRAS* expression in human cell lines can vary. In regards to cell lines utilized in this current study, H1299 show basal expression of wild-type and A549 express a mutant *KRAS.G12S*. The goal of the current project is to overexpress *KRAS* G12C in these two cell lines that harbor other sequences of KRAS in order to better understand the contribution of this mutation to the behavior of cancer cells. In order to do this, a KRAS construct was built that would also confer hygromycin resistance to cells that incorporate the sequence into their genomes. The mechanism of Hygromycin functions as an antibiotic by binding to the A site of the peptide transferase complex, preventing tRNA from progressing to P site, and effectively disrupting peptide formation (Kaminishi et al., 2015). Hygromycin would therefore provide a selective pressure for transgenic cells. Stable KRAS-G12C cancer cells could then be used to investigate altered sensitivity to standard-of-care chemotherapeutics, such as paclitaxel, cisplatin, and doxorubicin.

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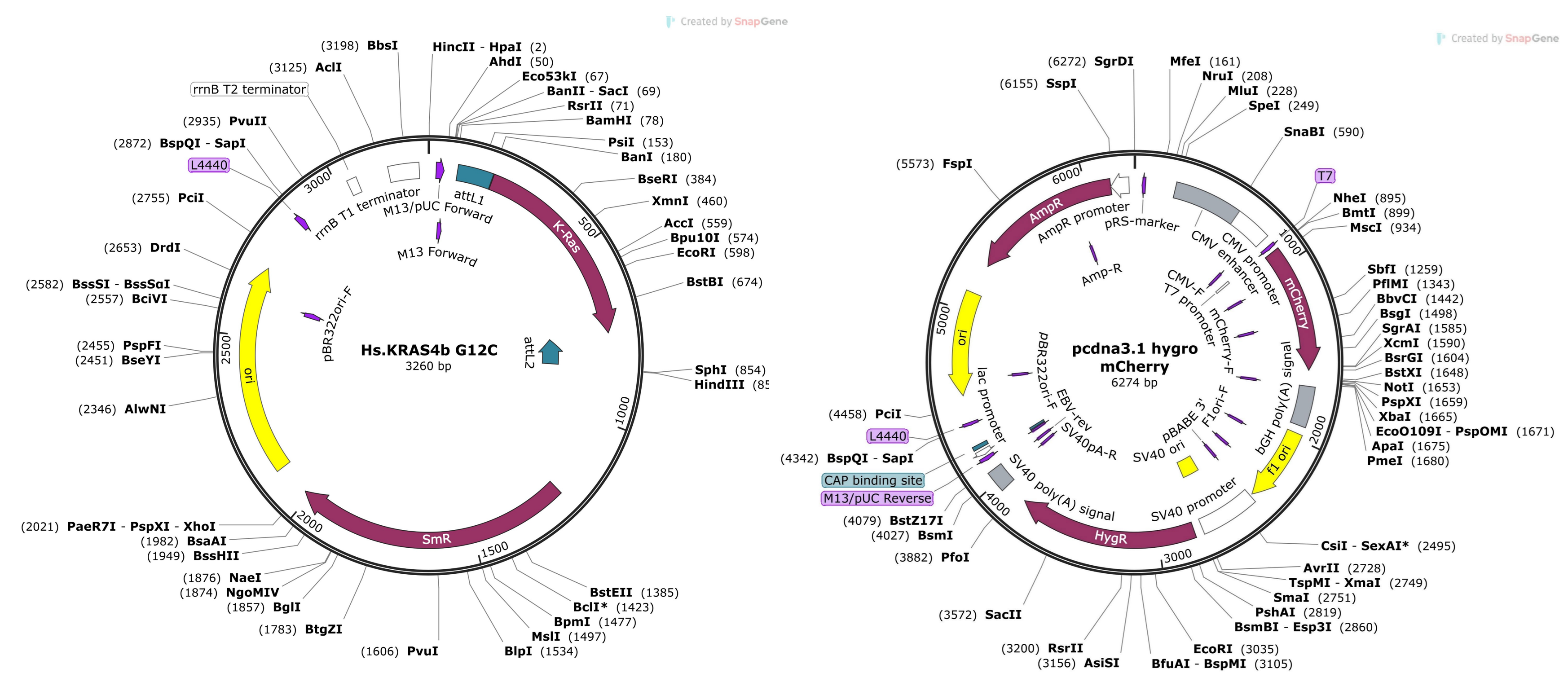
Materials and Methods

- Primers designed for forward and reverse sequences of Hygromycin and KRAS for amplification
- PCR of mutated *KRAS* to add restriction sites determined from AddGene (**Figure 1**)
- Clean and Concentrate of mutated *KRAS* PCR to amplify region of interest and eliminate unneeded components (**Figure 2**)
- Digests of mutated *KRAS* PCR and Hygromycin with NotI and NheI restriction enzymes individually to prepare for ligation
- Clean and Concentrate of Hygromycin-containing plasmid (**Figure 3**) and *KRAS* PCR of amplified gene of interest (**Figure 4**)
- Ligation of KRAS PCR into Hygromycin resistant cassette with ligation factor (**Figure 5**)
- Patched putative transformant Hygro/p*KRAS* constructs (**Figure 6**)
- Plasmid prep of putative transformant Hygro/p*KRAS* (**Figure 7**)
- PCR conformation of transformant p*KRAS*/Hygro construct is present in putative transformant (**Figure 8**)
- Western Blot to show baseline level of expression of KRAS G12C in A549 and H1299 cell lines (**Figure 9**)
- IC50 and MTT Survival Assay to determine antibiotic resistance for future transfection of Hygro/pKRAS into cell lines(**Figures 10A, 10B, 11A, 11B**)



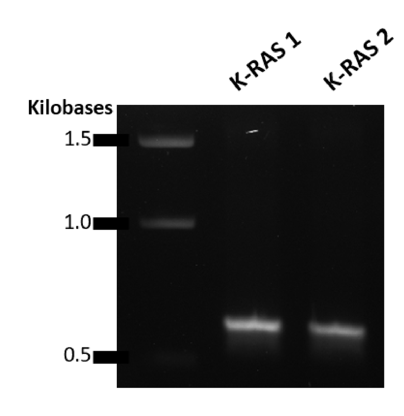


Figure 1. KRAS amplification

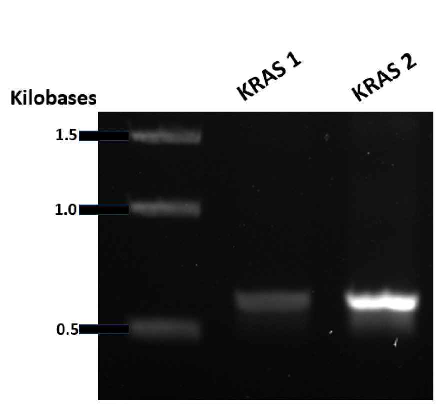


Figure 2. PCR of Clean & Concentrate KRAS

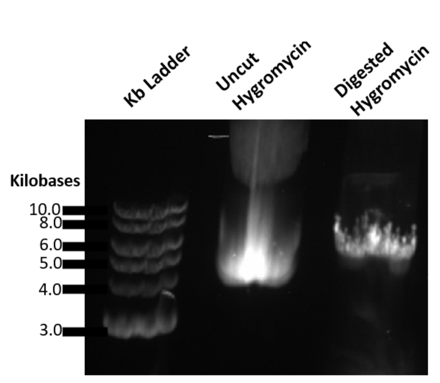


Figure 3. PCR of Clean and Concentrate Digested Hygro

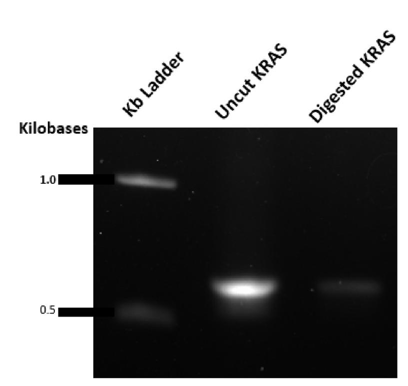


Figure 4. PCR of Clean and Concentrate Digested KRAS

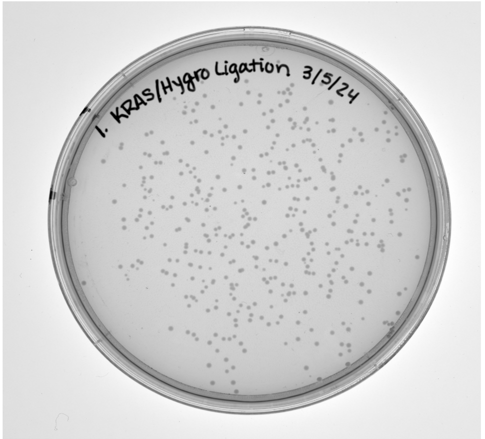


Figure 5. Ligation of KRAS into Hygro-containing Plasmid

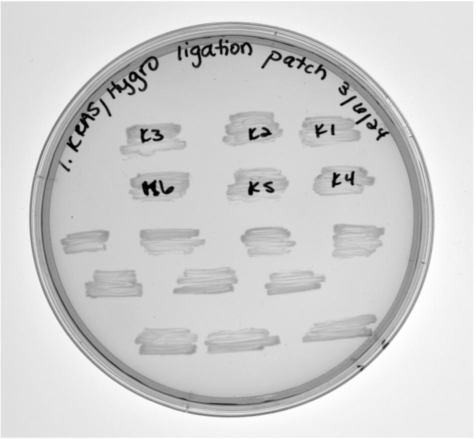


Figure 6. Patch Plate of Putative Transformative KRAS/Hygro Plasmid Constructs

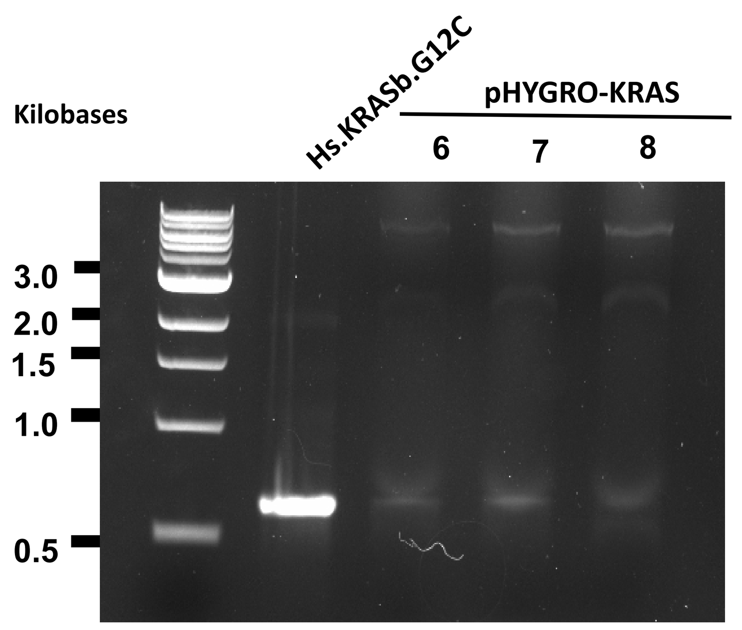


Figure 8. PCR of putative KRAS transformants

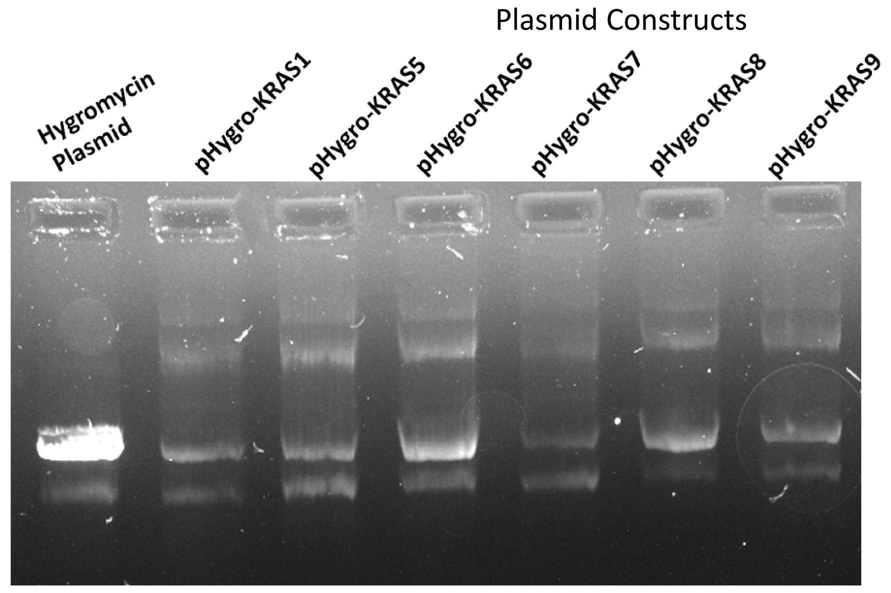


Figure 7. Size comparison of putative pHygro-KRAS to empty pHygro

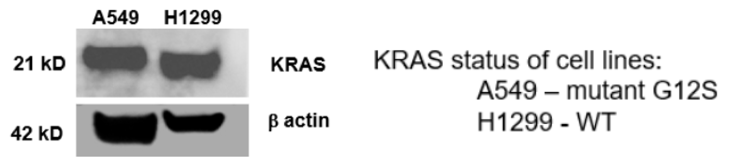
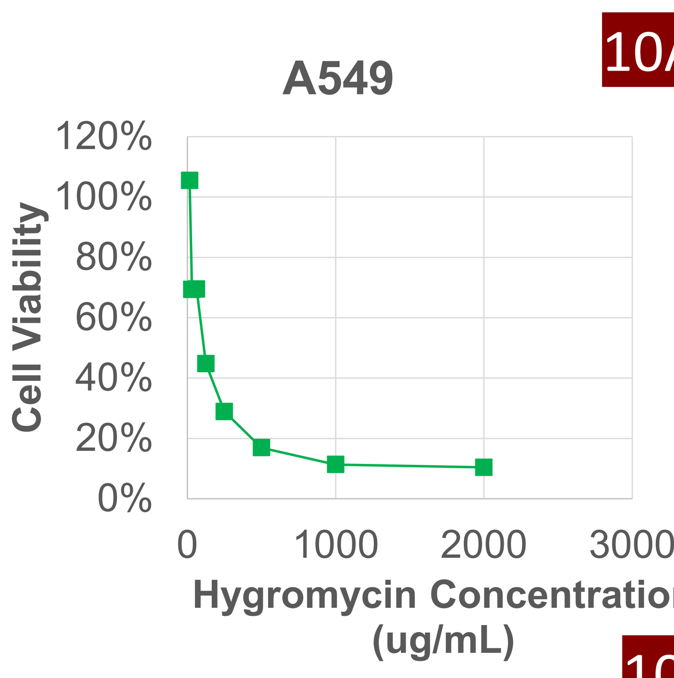


Figure 9. Western Blot results in comparison to β actin as a control. A549 cell lines inherently contain mutant of KRAS G12S and H1299 cells contain the wild-type allele of KRAS



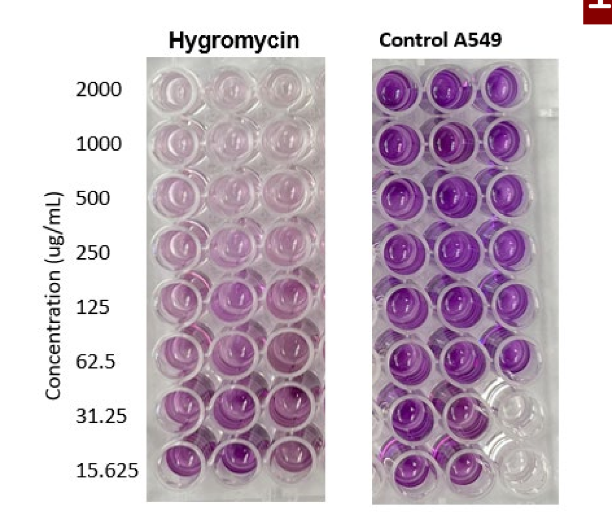
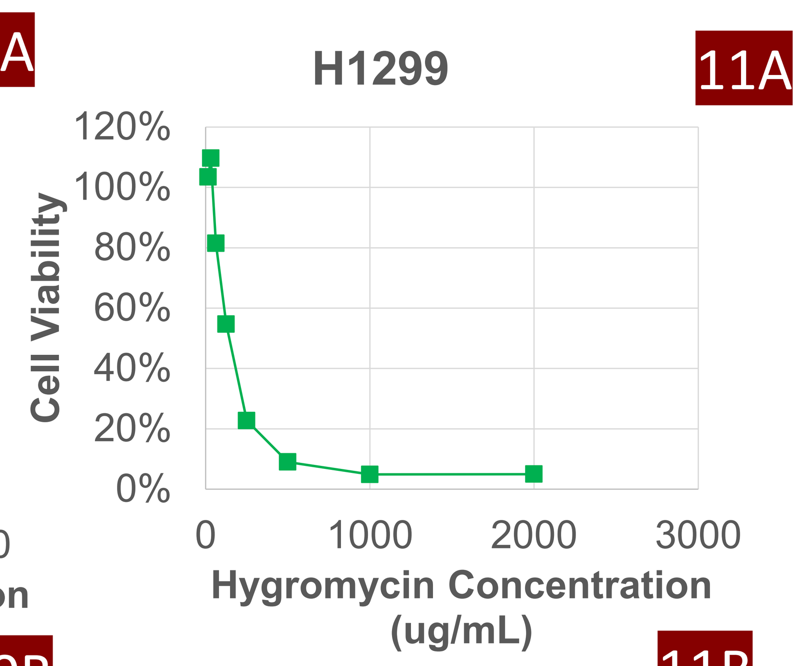


Figure 10A & 10B. A549 cells were plated in a 96-well plate (3000 cells per well). Cells were treated with 8 concentrations of hygromycin from 2 mg/mL to 15.625 mg/mL for 92 hours. After treatment, cells were incubated with MTT reagent and absorbance measured to assess cell survival. Survival of treated cells was calculated as a percentage absorbance of untreated cells.



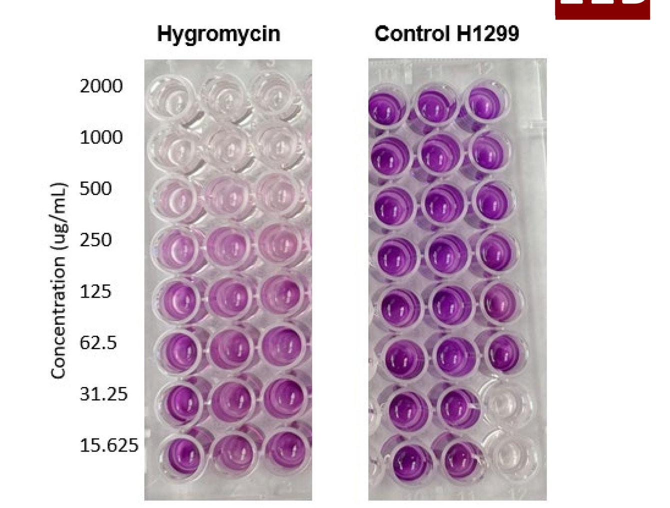


Figure 11A & 11B. H1299 cells were plated in a 96-well plate (5000 cells per well). Cells were treated with 8 concentrations of hygromycin from 2 mg/mL to 15.625 mg/mL for 92 hours. After treatment, cells were incubated with MTT reagent and absorbance measured to assess cell survival. Survival of treated cells was calculated as a percentage absorbance of untreated cells.

Discussion

- Constructed plasmid by amplifying gene from one source and placed into plasmid with antibiotic resistance
- IC50 determined to be 250ug/mL Hygromycin in A549 cell lines and 125ug/mL Hygromycin in H1299 cell lines
- Background level of gene in cells
- Western blot confirms KRAS expression within cell lines

Future Directions

- Transfection of human lung cancer cell lines A549 and H1299
- Presence of the G12C KRAS sequence will be confirmed by having the plasmid sequenced
- Transient expression of *KRAS* in cell lines
- Western Blot with antibodies for mutant *KRAS* to confirm functional protein
- Stable expression of KRAS in cell lines to use in chemotherapeutic drug effectiveness analysis

Acknowledgements

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- Dr. Gupta from University of Louisville for donation of human cell lines A549 and H1299.
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