Dissertation Prospectus on KRAS

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Describe the process for writing your prospectus and how it got from initial idea to final form.

The prospectus is a written summary of your proposed dissertation work typically required by your department during your 3rd year at Yale. This document is an updated version of the research proposal submitted during your qualifying exam and it follows the format of a NIH NRSA F31 application. In other words, your prospectus will have a 1-page summary of your research proposal (Specific Aims) followed by a detailed proposal (Research Strategy) which includes an overview of the relevant work in the field (Background), a description of how the proposed work will advance the field (Significance), and an exhaustive description of how the research will be conducted (i.e., methodology, controls, and analyses). While writing my prospectus, I revisited the written portion of my qualifying exam and incorporated the feedback I had received from my committee members. In addition, I was fortunate to co-write a pilot grant with my advisor prior to my prospectus. This opportunity allowed me to think of my project more critically and generate supporting figures of the workflow and preliminary data. These documents served as a solid foundation for my prospectus and allowed me to craft a refined research proposal that I repurposed for my NIH NRSA F31 fellowship application.

What advice would you give to current grad students writing their prospectuses?

If you are in the earlier stages of writing, I would advise you to jot down the key studies and preliminary data that serve as the basis of your proposed work. This will help you determine how you would like to frame your introduction/background section. Remember to explicitly state the gap in knowledge or problem your thesis work is addressing. If you find yourself struggling with where to start, you can always schedule a brainstorming session with a Graduate Writing Lab (GWL) fellow. Remember that GWL fellows can support you at various stages of the writing process and help you plan a writing schedule. Make sure to leverage the numerous resources you have at hand when writing your prospectus. For instance, your thesis advisor and thesis committee members are a great asset when it comes to evaluating the scientific merit of the proposed work and identifying relevant literature. They are experienced scientists that can help you elevate your proposal and improve your writing. Also, ask your friends and lab mates whether they can read your proposal. This can help improve the readability and accessibility of the document. Prospectuses are meant to be written in clear, plain English with little jargon. As you polish your proposal, remember you can obtain input from your advisor and schedule additional appointments with the GWL.

Specific Aims

KRAS, the most common oncogenic driver in human cancer, is mutated in >90% of human pancreatic ductal adenocarcinoma (PDAC) tumors1 and encodes for a small GTPase that regulates cell proliferation, metabolism, migration, and survival. Activating mutations in amino acids G12, G13, and Q61 prevent the induction of GTP hydrolysis by GTPase activating proteins (GAPs) and result in aberrant downstream signaling2. The prevalence of these mutants varies depending on the cancer type and they incongruously influence patient outcome and response to therapy3-6. Previous work characterizing the biochemical properties of these mutant KRAS oncoproteins revealed distinct intrinsic and extrinsic GTP cycling rates and altered binding affinities to effector proteins, supporting divergent signaling pathway activation7,8. Moreover, stark differences in their tumor initiation capacities have been observed in vivo and might explain the discordant prevalence of these mutations in human cancer5,9. However, it remains unclear the extent to which these biochemical differences alter signaling pathway activation and influence their differential tumorigenic competency. A better understanding of KRAS mutant-specific biology could help tailor therapeutic strategies to improve patient response and treatment outcome.

The long-term goal of this research is to understand the differences in signaling and tumorigenic potential between KRAS variants to identify allele-specific therapeutic targets. Our central hypothesis is that the biochemical differences between KRAS variants result in differential signaling pathway engagement, affecting cellular behavior and tumorigenic properties. This hypothesis is based on our initial observations that KRAS mutants activate canonical signaling pathways differently. Previous work by our lab led to the development of KRAS-deficient PDAC cell lines which survived CRISPR/Cas9-mediated KRAS ablation through adaptive signaling rewiring10. Both the phenotypic and signaling changes observed were reversed upon re-expression of the mutant KRAS variant of parental cells, affording a powerful isogenic system for comprehensive comparative analyses of KRAS variants in a relevant cancer cell model. I propose to leverage this system to dissect the differences and similitudes among KRAS variants in signaling network engagement and biological fitness in vitro and in vivo.

Aim 1: Dissect the differences in signaling networks between KRAS mutant variants

We hypothesize that identified biochemical differences between KRAS mutants will result in differential pathway engagement that define their sensitivity and response to treatment6,11. To test this hypothesis, I will express a large panel of KRAS variants found across human cancers at defined levels in KRAS deficient human PDAC cells. I will analyze global protein and phosphoprotein abundance by data-independent acquisition mass spectroscopy (DIA-MS) and correlate these to their respective transcriptional profiles obtained by RNA sequencing. Using supervised and unsupervised methods, I will cluster KRAS variants by their phosphoproteomic and gene expression profiles to identify specific signaling pathways engaged byspecific variants or groups of variants and correlate signaling to previously reported biochemical properties of individual KRAS variants 7,8.

To validate our findings in additional cancer models, I will perform western blotting and immunohistochemistry on autochthonous mouse PDAC tumors and cell lines, human PDAC resections, and PDAC PDX models encompassing a diverse set of KRAS mutants. Finally, I will test whether pharmacologic pathway inhibitors have variant-specific effects based on predicted vulnerabilities from phosphoproteomic data.

Aim 2: Compare the effects of KRAS mutants on cellular fitness in vitro and in vivo.

We hypothesize KRAS variants associated with worse prognosis in human cancer will demonstrate enhanced cellular proliferation in vitro, tumorigenic growth in vivo, and metastatic capacity. To test this hypothesis, I will use the re-expressed KRAS mutant variant and the CellTag system12 as genetic barcodes allowing sequence-based multiplexed quantification of individual KRAS-expressing cells in a pooled population. We will compare biological fitness by competitive proliferation assays in vitro and by monitoring tumorigenic growth and metastatic capacity in subcutaneous or orthotopic transplant in vivo. We will further test whether pharmacologic inhibition of signaling molecules upstream (SHP2i, SOS1i, EGFRi) or downstream (MEKi, PI3Ki) of KRAS alter cellular fitness both in vitro and in vivo.

Findings from this proposal will provide key insights into the molecular mechanisms underlying allele-specific KRAS biology. Furthermore, understanding the breadth of the differences in signaling and biological fitness of KRAS mutants could help identify unique dependencies that could be exploited to generate effective, allele-specific therapeutic interventions in PDAC and other KRAS mutant cancers.

Background and Significance

KRAS is the most frequently mutated oncogene and accounts for 23% of human cancers. This small GTPase act as signal transducer to regulate cellular growth, metabolism, migration, and survival. In homeostatic conditions, GTPases normally cycle between their active and inactive states through their modulation by guanine nucleotide exchange-factors (GEFs) and GTPase-activating proteins (GAPs), respectively. Recurrent cancer-associated RAS mutations occur in amino acid residues G12, G13 and Q61 which are found in the catalytic domain and are important for GAP-mediated inactivation. Biochemical analyses of several KRAS mutant forms have revealed fundamental variant-specific differences in the GTP cycling7, upstream regulation13-16, and downstream signaling8. Furthermore, in vitro studies suggest these variant-specific biochemical properties influence their response to therapy.

When comparing the representation of KRAS mutations across all cancers, variants G12D, G12V, and G12C account for more than 70% of cases3. However, the frequency of specific KRAS mutant variants differs by cancer type. Moreover, tumors harboring different KRAS mutations differ in prognosis in different cancer types4,5. To date, it has remained largely unclear why differences in mutant variant prevalence and outcomes are observed. Recent in vivo analysis of their tumor initiation capacities in pancreatic ductal adenocarcinoma (PDAC) mouse models demonstrated that KRAS mutant variants display differences in oncogenic potential17,18. Beyond their different effects in tumor pathogenesis, KRAS mutant variants may diverge in their functional roles in tumor maintenance, however a comprehensive comparative analysis of KRAS mutant variants in a cancer context is lacking. Previous work evaluated the biochemical and functional consequences of expressing KRAS mutants in isogenic non-transformed “normal” cell lines (e.g. 3T3 fibroblasts or human mammary

epithelial cells)11. While comprehensive, the relevance and interpretation of the results of these studies were confounded by differences in KRAS expression levels and the use of non-cancerous cell lines. Recent efforts exploring KRAS allele-specific biology in more faithful models, such as those incorporating endogenous KRASG12D and KRASA146T expression in the murine colon and pancreas, revealed discordant regulation of signaling pathways by phosphoproteomics but was limited by the scope of mutations being evaluated.12. Therefore, a systematic and comprehensive evaluation of allele-specific signaling networks in a cancer-relevant context is needed to better understand KRAS diversity and reveal variant-specific dependencies.

Prior work by our lab resulted in the generation of KRAS-deficient human PDAC cell lines10. These cells showed an adaptive yet reversible rewiring of signal transduction through the PI3K pathway. This work provided us with a useful isogenic system to compare the effect KRAS variants have on pathway engagement and their susceptibilities to regulation. The goal of the proposed project is to understand the effect of specific KRAS mutations on signal transduction and cellular transcriptome to identify potential pathway dependencies. Also, this work will evaluate their impact on cellular fitness and tumorigenic capacities. For this, I will study the divergence in signaling network engagement between KRAS variants of the most frequently mutated amino acids by comparing their transcriptomic, proteomic and phosphoproteomic profiles. Additionally, I will evaluate the cellular fitness of KRAS variants and their response to perturbations both in vitro and in vivo using a multiplex-approach. I hypothesize that the biochemical differences between these variants induce a permissive cellular state that deems certain mutants more fit to establish tumors. Most of the work will be carried out at West Campus between the Muzumdar and Liu laboratories. Also, we will use the Yale Center for Genome Analysis (YCGA) as a sequencing resource.

Elucidating the mechanisms involved in oncogenic RAS signaling and their biological consequences could translate to the development of new treatments for what was once considered an undruggable target. This work will provide information on how these variants might rely on canonical or non-canonical pathways to promote tumor formation and metastasis. Furthermore, it will help clarify the differences in drug response based on KRAS G12, G13 and Q61 variants and their activity levels. Lastly, this work will provide insight on how mutant-specific signaling networks might influence their cellular competency and affect tissue colonization.

Experimental Plan

Preliminary Data: KRAS variants activate canonical signaling pathways differently. To compare KRAS allele-specific differences in phosphorylation events, we re-expressed a subset of KRAS variant cDNAs by lentiviral transduction in KRAS deficient cells (Fig. 1A). I used a co-expressed fluorescent reporter (mCherry via a self-cleaving 2A peptide) to sort cells that stably expressed KRAS at specific levels (Fig. 1B). Preliminary data from these studies revealed divergence in baseline signaling through the canonical MAPK and PI3K pathways (Figs. 1C-F), highlighting how KRAS variants differ in signaling output in PDAC cells. At increasing KRAS expression levels, the mutants evaluated had a dose-dependent increase in both pERK and pAKT levels. Interestingly, G12D and G12C mutants showed strong engagement of both signaling pathways. These results could potentially explain the strong oncogenic potential seen in PDAC mouse models and their prevalence in the clinic.

Aim 1: Dissect differences in signaling networks between KRAS mutant variants

In this Aim, we propose a comprehensive phosphoproteomic and transcriptomic analysis to define alterations in signaling networks based on KRAS allele. We hypothesize that the biochemical differences between KRAS variants identified in previous studies5,6 will result in differential pathway engagement that define their sensitivity and response to treatment. Identifying allele-specific oncogenic signaling vulnerabilities could translate to novel therapeutic strategies in PDAC and other KRAS mutant cancers.

Aim 1.1: Generate transcriptional and phosphoproteomic profiles of KRAS variants Experimental

Approach: To compare KRAS allele-specific differences in phosphorylation events, I will re-express 15 unique KRAS variants in human KRAS-deficient PDAC cell lines by lentiviral transduction (Figs. 1A-B). I will use fluorescence-activated cell sorting (FACS) to control for KRAS protein expression levels and confirm oncogenic KRAS signaling by examining activation of canonical signaling pathways via western blot, using levels of phospho-ERK and phospho-AKT as a readout. Given that our preliminary data indicates KRAS mutants display the highest variability at medium expression levels of the oncoprotein (Figs. 1E-F), I will select this expression level to generate their respective phosphoproteomic, total proteomic, and transcriptional profiles15. By controlling for oncoprotein expression levels, we seek to distinguish allele-specific divergence in signaling independent of KRAS dose. In collaboration with Dr. Yansheng Liu's lab, we will examine the phosphoproteomic profiles (with matched total proteomic changes) of KRAS mutant cells in their respective steady states by DIA-MS, using their Orbitrap-Lumos platform and direct-DIA workflow16 that allows confident localization of 25,000-30,000 class I phosphopeptides. Their established phosphopeptide enrichment protocol using High-Select Fe-IMAC beads has achieved stable enrichment efficiency of >99% from ~150 µg of total peptides. In parallel to phosphoproteomics, I will generate transcriptional profiles by RNA sequencing (RNA-seq) by isolating total RNA from KRAS mutant cells and performing polyA-enriched library preparation and 75nt paired-end Illumina sequencing to a target 20M read count with the Yale Center for Genome Analysis (YCGA). Phosphoproteomic, total proteomic, and transcriptional profiles will then be correlated to build signaling networks for each KRAS variant across clones, as done by the Liu lab previously15. Specifically, I will perform unsupervised clustering analyses including hierarchical clustering, principal component analysis (PCA), or independent component analysis (ICA), an unbiased blind source separation method our lab has used previously13 to identify signals in variables of interest (e.g. KRAS variant) over noise introduced by differences between cell lines and clones. To test the feasibility of our approach, I reconstituted a subset of KRAS mutants into one of our KRAS-deficient cell lines and performed DIA-MS and RNA-seq. Our preliminary results show that we can successfully identify up to 44,191 phosphorylation sites with a 70-80% reliability, ensuring our methods are highly confident and highly sensitive. Furthermore, we were able to detect ~7,500 proteins which highly correlated in abundance to their respective mRNA transcripts (Fig. 2). No global differences in translation efficiency were observed among KRAS mutants.

Unsupervised hierarchical clustering of RNA-seq and phosphoproteomic data identified distinct groups of KRAS mutants in each dataset (Fig. 3), consistent with predicted divergent signaling and transcriptional output. Strikingly, the three most prevalent PDAC mutations (G12D, G12V, and G12R) clustered together across both analyses (Fig. 3), arguing for a potential optimal convergent cell state induced by these mutations in PDAC. To ensure our approach is rigorous and generalizable, I will expand this analysis to the full set of 15 variants and analyze two independent clones from two parental cell lines (Fig. 1A). For KRAS variants that cluster together (e.g. G12D, G12V, and G12R), I will compare their transcriptional profiles to all other KRAS variants using DESeq and goseg in RStudio to identify differentially expressed genes. In parallel, I will use the Metascape and Kinase-Substrate Enrichment Analysis (KSEA) algorithms to test for pathway enrichment and perform protein analysis networks of our proteomics and transcriptomic data. For signaling or transcriptomic features that are allele-specific or overlap in certain groups of variants, we will confirm them using alternative approaches (western blotting for phosphoproteins or qRT-PCR for gene expression) in our PDAC cell lines. Aim **1.2: Validation of signaling differences in orthologous murine and human model systems**

Experimental Approach: To ensure that the allele-specific transcriptomic and phosphoproteomic differences are robust, I will confirm them in additional models: (1) an isogenic series of murine PDAC models, (2) a panel of human PDX models, and (3) a banked collection of human PDAC tumor specimens. Specifically, I will generate Pdx1-Cre;KrasLSL-mut; p53LSL-R172H (KPC) mice by breeding our established Pdx1-Cre; p53LSL-R172H mice with a recently develop allelic series of KrasLSL-mut mice (G12D, G12C. G12R, and G13D)8. I will isolate PDAC tumor tissue from these mice to perform RNA FISH and immunohistochemistry (IHC) on FFPE sections. Also, I will perform qRT-PCR or western blot (WB) using available antibodies against peptides and phosphopeptides on PDAC cell lines estabished from these tumors. (Aim 1.1). Furthermore, I will perform the corresponding IHC and WB analyses in cell lines and tissue samples from patient-derived xenografts (PDX) models17,18 obtained from our collaborator Dr. Chen Liu and de-identified tumor ressections from the Yale GI Cancer Biobank facilitated by Drs. John Kunstman and Marie Robert (Fig. 4). These experiments will confirm that the transcriptomic and proteomic features can be recapitulated in orthologous mouse and human tissues.

Aim 1.3: Exploring signaling pathway dependencies of KRAS mutants

Experimental Approach: To determine whether variant-specific pathways identified by Metascape analyses of phosphoproteomic data (in Aim 1.1) predict dependencies, I will expose KRAS-reexpressing cells to available inhibitors targeting signaling pathways and gauge susceptibility to short-term (72 hrs; CellTiter-Glo for cell viability, Annexin V for apoptosis) or long-term (7-10d; colony-forming assay) treatment. Since variants have altered intrinsic GTP cycling rates and effector binding, I will further assess whether variants display different sensitivity to inhibitors of canonical upstream (SHP2, EGFR) and downstream (MEK, PI3K) signaling molecules.

Anticipated outcomes, potential pitfalls, and alternative strategies: We hypothesize that some alleles will show unique signaling networks, such as receptor tyrosine kinase (RTK) signaling. For example, KRASG13D colorectal cancer, unlike tumors harboring other KRAS mutant alleles, have been shown to respond to anti-EGFR antibodies19,20. We further hypothesize that KRASG12D, KRASG12V, and KRASG12R alleles might share overlapping signaling features compared to the others that might induce a permissive cellular state that underlies key PDAC pathways, given these three alleles constitute 90% of KRAS mutations in human PDAC21. Indeed, our preliminary data to date support this hypothesis and the feasibility of the approach (Figs. 2-3). Furthermore, our robust array of murine and human models will add rigor and robustness to our findings. A key limitation of our proteomic approach is in the limited detection of phosphotyrosine (pY) residues (including RTKs) due to their low abundance compared to phosphosites at serine or threonine residues. To enhance sensitivity for pY peptides, we can use a pY-specific antibody for enrichment prior to DIA-MS, as we have done previously. An alternative strategy would be to employ commercial pY kinome profiling kits (Cell Signaling), an antibody-based approach that allows comparing phosphorylation changes in a more limited set of proteins (including RTKs), possibly with greater sensitivity. Finally, our validation efforts may be limited by the availability of antibodies and inhibitors targeting specific phosphopeptides and pathways of interest, respectively, though RNA FISH methods would still allow direct abundance analyses of differentially expressed genes identified in transcriptomic and proteomic analyses.

Aim 2: Compare the effects of KRAS mutants on cellular fitness in vitro and in vivo.

The prevalence of KRAS mutants differs based on tumor type1. Studies comparing the effect KRAS genotype on patient survival further revealed that specific mutants were associated with poor prognosis depending on the cancer context6,7,9. To explain these clinical data, we hypothesize that KRAS mutants vary in their effects on cellular fitness in different environments. In this Aim, I will test this hypothesis by performing competitive functional assays to evaluate the effects of KRAS mutants on cell proliferation in vitro and tumorigenic growth, and metastasis in vivo.

Aim 2.1: Compare the effects of KRAS mutant variants on anchorage-dependent and -independent growth in vitro.

Experimental Approach: I propose to use a genetic barcoding system to allow multiplexed competitive fitness analysis of pooled cells harboring different variants. Each lentivirally-transduced KRAS variant serves as its own barcode that can be PCR amplified from genomic data isolated from these pools. Since it is possible that there may be discrepancies between the number of viral integration events and the level of mCherry-2A-KRAS expression that could confound this analysis, we will use a second genetic labeling system based on the CellTag (CT) system22 to ensure robust results.

These CT barcodes consist of a lentiviral GFP construct that contains a SV40 polyadenylation signal that precedes a unique 10-bp molecular identifier. The CT system allows long-term monitoring of mixed cellular populations including in competitive transplant experiments. In a multiplexed approach using sequence-based readout to detect differences in cellular fitness, I will co-transduce KRAS deficient cells with a specific KRAS mutant variant and its respective CellTag barcode and sort by mCherry and GFP fluorescence to confirm transduction (Fig. 5). Then, I will pool CT/KRAS mutant cells and growth them in anchorage-dependent (in 2D culture on plastic or organoid culture in Matrigel) and anchorage-independent (soft agar) conditions, as our lab has done previously13. We will collect genomic DNA (gDNA) from these cells at various timepoints and measure the relative number of cells harboring each variant by prolonged tracking of barcode abundance, allowing us to compare competitive fitness of KRAS mutants. To detect the barcodes, I will PCR amplify the KRAS variant and the CT barcode with Illumina sequencing adaptors for Nova-analysis in the Yale Center for Genome Analysis (YCGA). I will then use the CellTag construct or KRAS sequence as a reference to align my reads and track changes in the sequence representation over time. I have piloted the CT barcode system and can distinguish individual barcodes from cell pools harboring barcodes in <0.05% of cells, supporting the sensitivity of barcode detection. Using this approach, I will measure competitive fitness in vitro at baseline and following treatment with pharmacologic inhibitors of signaling molecules upstream or downstream of KRAS (as in Aim 1.3). As a positive control for this analysis, I will use recently developed KRAS G12C-specific inhibitors (e.g. ARS-1620), which would be expected to deplete KRAS G12C-expressing cells. Lastly, I will use correlative analyses to evaluate the correlation between baseline KRAS mutant biologic fitness (most fit vs non-fit mutants) and their signaling features to identify key pathways that might influence their competency. Aim 2.2: Evaluate tumorigenic and metastatic capacities in vivo using subcutaneous and orthotopic xenograft models.

Experimental Approach: Taking advantage of our CT barcoding system and data deconvolution strategy, I will evaluate the tumorigenic capacities of the different variants following transplantation into immunocompromised athymic nude mice. KRAS deficient cells co-expressing the CT barcode, individual KRAS variants, and luciferase reporter will be pooled together in equal numbers. 1M pooled cells will be subcutaneously injected in both flanks of 5 mice, resulting in at least 5 technical replicates (50% engraftment rate). I will measure tumor growth using calipers until the tumors reach ~0.5-1cm3, when mice will be euthanized, and tumors extracted for gDNA extraction using a Roche High-Capacity kit. Mouse lungs and liver will also be analyzed for metastases by bioluminescent imaging (IVIS), and gDNA will be extracted from metastases. Barcodes will be PCR amplified from gDNA samples and analyzed as described in Fig. 5. I will evaluate differences in barcode representation as an indication of their in vivo fitness both in the primary tumor and in the metastatic sites. I will also test whether treatment with pharmacologic inhibitors of signaling molecules upstream or downstream of KRAS (as in Aim 1.3) alter in vivo fitness. I will further perform orthotopic implants into the tail of the pancreas in ten (10) immunocompromised mice. Tumor growth will be tracked by in vivo bioluminescent imaging and/or MRI23. Our combined analysis of subcutaneous and orthotopic xenograft models and associated metastases will allow us to evaluate how the tumor environment might influence variant fitness24.

Anticipated outcomes, potential pitfalls, and alternative strategies: We expect that the most prevalent variants in PDAC (G12D, G12V, and G12R) will show higher representation in our in vitro and in vivo competition assays. We further do not anticipate any differences in Since of these G12D is associated with the worst prognosis in humans, we would further anticipate that G12D would have a competitive advantage to the other common variants and would be consistent with its more potent tumor initiation capacity in mice when compared to G12R and G13D8. Furthermore, we expect Q61 mutants to be more resistant to upstream regulators (e.g. SHP2 or EGFR inhibitors) given their lower GTP hydrolysis rates. One potential limitation of my multiplexed approach is the inability to distinguish if cooperativity between variants is leading to the fitness effects. To evaluate this, we will test in vitro proliferation and in vivo tumorigenic growth rates of the variants of interest individually.

Dissertation Timeline

Activity	Academic Year
Aim 1.1 Generate transcriptomic and proteomic profiles of PDAC cells	***Spring 2021***
Aim 2.1. Generate CellTag indexed isogenic cells	***Spring 2021***
2nd Thesis Committee Meeting	***Spring 2021 (May)***
Aim 2.1. Competitive fitness assays *in vitro* +/- drug inhibitors	***Fall 2021***
Aim 1.2. Breed *KPC* mice for model generation	***Fall 2021***
3rd Thesis Committee Meeting	***Fall 2021 (December)***
Aim 1.2. Validate allele-specific signaling in isogenic *KPC* models	***Spring 2022***
Aim 2.2. Competitive fitness *in vivo* +/- drug inhibitors in athymic mice	***Spring 2022***
4th Thesis Committee Meeting	***Spring 2022 (May)***
Aim 1.2. Validate allele-specific signaling in PDX models and tumor resections	***Fall 2022- Spring 2023***
5th Thesis Committee Meeting	***Fall 2022 (December)***
Aim 1.3. Explore signaling pathway dependences in *KRAS* mutant cells	***Spring 2023***
6th Thesis Committee Meeting	***Spring 2023 (May)***
Write thesis	***Fall 2023***
Prepare and submit 1-2 manuscripts	***Fall 2023- Spring 2024***
Defend PhD dissertation	***Spring 2024***

References

1 (TCGA), T. C. G. A. R. N. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. *Cancer cell* **32**, doi:10.1016/j.ccell.2017.07.007 (2017).

2 Scheffzek, K. *et al.* The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science* **277**, 333-338, doi:10.1126/science.277.5324.333 (1997).

3 Cosmic. *COSMIC - Catalogue of Somatic Mutations in Cancer*, <https://cancer.sanger.ac.uk/cosmic> (2019).

4 Nadal, E. *et al.* KRAS-G12C mutation is associated with poor outcome in surgically resected lung adenocarcinoma. *J Thorac Oncol* **9**, 1513-1522, doi:10.1097/jto.0000000000000305 (2014).

5 Bournet, B. *et al.* KRAS G12D Mutation Subtype Is A Prognostic Factor for Advanced Pancreatic Adenocarcinoma. *Clin Transl Gastroenterol* **7**, e157, doi:10.1038/ctg.2016.18 (2016).

6 D, R. *et al.* KRAS G13D sensitivity to neurofibromin-mediated GTP hydrolysis. *Proceedings of the National Academy of Sciences of the United States of America* **116**, doi:10.1073/pnas.1908353116 (2019).

7 Hunter, J. C. *et al.* Biochemical and Structural Analysis of Common Cancer-Associated KRAS Mutations. *Mol Cancer Res* **13**, 1325-1335, doi:10.1158/1541-7786.mcr-15-0203 (2015).

8 Ihle, N. T. *et al.* Effect of KRAS oncogene substitutions on protein behavior: implications for signaling and clinical outcome. *J Natl Cancer Inst* **104**, 228-239, doi:10.1093/jnci/djr523 (2012).

9 Zafra, M. P. *et al.* An in vivo KRAS allelic series reveals distinct phenotypes of common oncogenic variants. *Cancer Discovery* **10**, 1654-1671, doi:10.1158/2159-8290.CD-20-0442 (2020).

10 Muzumdar, M. D. *et al.* Survival of pancreatic cancer cells lacking KRAS function. *Nature Communications* **8**, 1-19, doi:doi:10.1038/s41467-017-00942-5 (2017).

11 McFall, T. *et al.* A systems mechanism for KRAS mutant allele–specific responses to targeted therapy. doi:10.1126/scisignal.aaw8288 (2019).
12 Guo, C. *et al.* CellTag Indexing: genetic barcode-based sample multiplexing for single-cell genomics. *Genome Biol* **20**, 90, doi:10.1186/s13059-019-1699-y (2019).
13 Chen, Y. N. *et al.* Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. *Nature* **535**, 148-152, doi:10.1038/nature18621 (2016).
14 Mainardi, S. *et al.* SHP2 is required for growth of KRAS-mutant non-small-cell lung cancer in vivo. *Nat Med* **24**, 961-967, doi:10.1038/s41591-018-0023-9 (2018).
15 Nichols, R. J. *et al.* RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. *Nat Cell Biol* **20**, 1064-1073, doi:10.1038/s41556-018-0169-1 (2018).
16 Ruess, D. A. *et al.* Mutant KRAS-driven cancers depend on PTPN11/SHP2 phosphatase. *Nat Med* **24**, 954-960, doi:10.1038/s41591-018-0024-8 (2018).
17 Poulin, E. J. *et al.* Tissue-Specific Oncogenic Activity of KRAS(A146T). *Cancer Discov* **9**, 738-755, doi:10.1158/2159-8290.cd-18-1220 (2019).
18 Zafra, M. P. *et al.* An in vivo KRAS allelic series reveals distinct phenotypes of common oncogenic variants. *bioRxiv*, 847509, doi:10.1101/847509 (2019).
19 Janes, M. R. *et al.* Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. *Cell* **172**, 578-589.e517, doi:10.1016/j.cell.2018.01.006 (2018).
20 Lou, K. *et al.* KRAS(G12C) inhibition produces a driver-limited state revealing collateral dependencies. *Sci Signal* **12**, doi:10.1126/scisignal.aaw9450 (2019).
21 Misale, S. *et al.* KRAS G12C NSCLC Models Are Sensitive to Direct Targeting of KRAS in Combination with PI3K Inhibition. *Clin Cancer Res* **25**, 796-807, doi:10.1158/1078-0432.ccr-18-0368 (2019).
22 Xue, J. Y. *et al.* Rapid non-uniform adaptation to conformation-specific KRAS(G12C) inhibition. *Nature* **577**, 421-425, doi:10.1038/s41586-019-1884-x (2020).
23 Ahmed, T. A. *et al.* SHP2 Drives Adaptive Resistance to ERK Signaling Inhibition in Molecularly Defined Subsets of ERK-Dependent Tumors. *Cell Rep* **26**, 65-78.e65, doi:10.1016/j.celrep.2018.12.013 (2019).
24 Simanshu, D. K., Nissley, D. V. & McCormick, F. RAS Proteins and Their Regulators in Human Disease. *Cell* **170**, 17-33, doi:10.1016/j.cell.2017.06.009 (2017).
25 Hung, V. *et al.* Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2. *Nat Protoc* **11**, 456-475, doi:10.1038/nprot.2016.018 (2016).
26 Lambert, J. M. *et al.* Tiam1 mediates Ras activation of Rac by a PI(3)K-independent mechanism. *Nat Cell Biol* **4**, 621-625, doi:10.1038/ncb833 (2002).
27 Lim, K. H. *et al.* Divergent roles for RalA and RalB in malignant growth of human pancreatic carcinoma cells. *Curr Biol* **16**, 2385-2394, doi:10.1016/j.cub.2006.10.023 (2006).
28 Lu, S., Jang, H., Nussinov, R. & Zhang, J. The Structural Basis of Oncogenic Mutations G12, G13 and Q61 in Small GTPase K-Ras4B. *Scientific Reports* **6**, 21949, doi:10.1038/srep21949 (2016).
29 Scheele, J. S., Rhee, J. M. & Boss, G. R. Determination of absolute amounts of GDP and GTP bound to Ras in mammalian cells: comparison of parental and Ras-overproducing NIH 3T3 fibroblasts. *Proc Natl Acad Sci U S A* **92**, 1097-1100, doi:10.1073/pnas.92.4.1097 (1995).

30 Chen, C. L. & Perrimon, N. Proximity-dependent labeling methods for proteomic profiling in living cells. *Wiley Interdiscip Rev Dev Biol* **6**, doi:10.1002/wdev.272 (2017).
31 Han, S., Li, J. & Ting, A. Y. Proximity labeling: spatially resolved proteomic mapping for neurobiology. *Curr Opin Neurobiol* **50**, 17-23, doi:10.1016/j.conb.2017.10.015 (2018).
32 Duncan, J. S. *et al.* Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. *Cell* **149**, 307-321, doi:10.1016/j.cell.2012.02.053 (2012).
33 Vaseva, A. V. *et al.* KRAS Suppression-Induced Degradation of MYC Is Antagonized by a MEK5-ERK5 Compensatory Mechanism. *Cancer Cell* **34**, 807-822.e807, doi:10.1016/j.ccell.2018.10.001 (2018).
34 Carceles-Cordon, M. R.-F., Irene.Rodriguez-Bravo, Veronica. Cordon-Cardo, Carlos. Domingo-Domenech, Josep. *In vivo Bioluminescence Imaging of Luciferase-labeled ...* <https://bio-protocol.org/e1762> (2016).
35 Rogers, Z. N. *et al.* A quantitative and multiplexed approach to uncover the fitness landscape of tumor suppression in vivo. *Nat Methods* **14**, 737-742, doi:10.1038/nmeth.4297 (2017).
36 Macosko, E. Z. *et al.* Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. *Cell* **161**, 1202-1214, doi:10.1016/j.cell.2015.05.002 (2015).
37 Zilionis, R. *et al.* Single-cell barcoding and sequencing using droplet microfluidics. *Nat Protoc* **12**, 44-73, doi:10.1038/nprot.2016.154 (2017).
38 Santana-Codina, N., Mancias, J. D. & Kimmelman, A. C. The Role of Autophagy in Cancer. *Annu Rev Cancer Biol* 1, 19-39, doi:10.1146/annurev-cancerbio-041816-122338 (2017).
39 Satoh, K., Hamada, S. & Shimosegawa, T. Involvement of epithelial to mesenchymal transition in the development of pancreatic ductal adenocarcinoma. *J Gastroenterol* **50**, 140-146, doi:10.1007/s00535-014-0997-0 (2015).
40 Kinsey, C. G. *et al.* in *Nat Med* Vol. 25 861 (2019).