

Research Training Plan

Defining Contributions of Pancreatic Ductal and Acinar Cells to Tumorigenesis

A. Specific Aims

The cellular origin of pancreatic tumors remains a vital topic for research to further our understanding of carcinogenesis. We currently do not know if cellular origin determines the phenotype of pancreatic tumors. Ninety percent of newly diagnosed pancreatic cancers are ductal adenocarcinomas (PDA). The majority of PDA are thought to originate from low-grade precursor tumors termed pancreatic intraepithelial neoplasms (PanIN) that progress into invasive adenocarcinoma in a step-wise fashion as they acquire more mutations [1].

Mutation in the k-ras gene is one of the earliest genetic alterations in PDA and an activating mutation in k-ras (k-ras^{G12D}) expressed in mouse pancreata recapitulates the human disease and produces PanIN and PDA. Concomitant mutation in the tumor suppressor Trp53 (Trp53^{R172H}), the mouse ortholog of one of the most common p53 mutations found in human PDA, accelerates disease progression [2]. Because PDA resemble normal ductal epithelium histologically (cuboidal shape, ductal surface antigens, and tubular growth), duct cells are often assumed to be the source of carcinogenesis. Furthermore, studies have also shown that expressing mutations of key genes that lead to PDA throughout the pancreas results in only ductal tumor formation [3].

However, there is evidence to suggest that PDA arise from other cell types. Acinar-to-ductal metaplasia is often associated with malignancy, so acinar cells are a potential source of PDA. This possibility is supported by a recent study showing that a different form of activated k-ras (k-ras^{G12V}) restricted to pancreatic embryonic cells of acinar and centroacinar lineage in adult mice led to PanIN and PDA formation in the setting of chronic pancreatitis [4]. In contrast, previous studies that have targeted k-ras^{G12D} to acinar cells found lethal tumors of mixed histology, rather than PanIN/PDA [5]. However, these mutations were expressed through all developmental phases, so we do not know the effects of inducing k-ras^{G12D} in only adult acinar cells. We also do not know the effects of k-ras^{G12D} mutation on adult duct cells because we lack the promoters to express mutated alleles specifically into that cell population. The first goal of my proposal is to test if adult pancreatic acinar or ductal cells that express k-ras^{G12D}/ Trp53^{R172H} can give rise to PDA.

We have recently demonstrated that Wnt signaling is activated in the majority of human PDA and in mouse models of PDA based on k-ras activation [6]. These results imply that Wnt signaling may be a downstream response to k-ras deregulation that leads to pancreatic carcinogenesis. To determine whether Wnt activation in the absence of k-ras mutations is sufficient to cause PDA, we ectopically expressed an activated form of β -catenin (β -cat^{ex3}), a downstream effector of Wnt, in the pancreas. We found that when β -cat^{ex3} was expressed to acinar and islet cells there was only pancreatic hyperplasia. However, when we used a promoter whose expression included the ductal compartment (along with acinar and islet cells) the transgenic mice surprisingly developed solid pseudopapillary tumors (SPT), a rare and indolent type of pancreatic tumor [7]. SPT have not been studied in detail, though we do know a great majority of these lesions harbor activating β -catenin mutations in human samples [8]. As is true with PDA, we do not know which cell type gives rise to these SPT. Our results do suggest that SPT arise from duct cells and imply that cellular origin of pancreatic tumors plays a central role in determining pancreatic tumor phenotype. Therefore, the β -cat^{ex3}-SPT model is a useful tool for exploring this hypothesis in further detail. The second goal of my proposal is to test if adult pancreatic acinar or ductal cells that express β -cat^{ex3} can give rise to SPT.

In summary, the goals of my proposal are to test the ability of pancreatic acinar and ductal cells to give rise to PDA and SPT. **My hypothesis is that the cellular origin of pancreatic tumors is an important factor in determining pancreatic tumor phenotype.** This hypothesis will be tested in two specific aims:

Specific Aim 1: To determine the role of adult pancreatic acinar cells (AC) in the development of pancreatic ductal adenocarcinomas (PDA) and solid pseudopapillary tumors (SPT).

Rationale: There is conflicting evidence about the cellular origin of PDA. Studies have suggested that AC may be the source of PDA via metaplasia. SPT are not well-studied and our novel mouse model of this disease implies that these lesions arise from DC. However, the results are not definitive. By expressing tumorigenic mutations that lead to these lesions (k-ras^{G12D}/Trp53^{R172H} for PDA and β -cat^{ex3} for SPT) in only AC, I will investigate if AC can give rise to PDA and/or SPT.

- A. Cross transgenic mice that carry conditionally expressed k-ras^{G12D}/Trp53^{R172H} or β -cat^{ex3} to mice that express tamoxifen-dependent Cre recombinase under the control of the elastase gene promoter (Ela-CreER), thus targeting mutations to only AC.
- B. Inject tamoxifen in adult mice to induce Cre recombination-excision of the floxed stop signal to activate the given mutations in adult pancreata, thus bypassing the effects of β -cat^{ex3} in developing pancreata.
- C. Observe mice for tumor development and characterize lesion phenotype via gross morphology, histologic characteristics, and immunohistochemical markers. Gene profiles will also be established with quantitative PCR.

Specific Aim 2: To determine the role of adult pancreatic ductal cells (DC) in the development of PDA and SPT.

Rationale: Because of the conflicting data about the cellular origin of PDA, I will also test if expressing k-ras^{G12D}/Trp53^{R172H} in DC can cause PDA formation. Similarly, by expressing β -cat^{ex3}, I will study if SPT can arise from DC. Because there is currently no promoter that allows for specific gene expression into DC *in situ*, I will adopt a different strategy than the one used in Specific Aim 1A. DC will be isolated and transformed *in vitro* and then reimplanted into nude mice for observation for tumor formation.

- A. Isolate and culture DC from adult mice that carry conditionally expressed k-ras^{G12D}/Trp53^{R172H} or β -cat^{ex3}.
- B. Introduce Cre recombinase and luciferase to cultured cells via lentivirus.
- C. Re-implant transformed cells into nude mice and monitor for tumorigenesis. Through luminescence, luciferase will allow for *in vivo*, real-time evaluation of metastasis.
- D. Characterize lesion phenotype via gross morphology, histologic characteristics, and immunohistochemical markers. Gene profiles will also be established with quantitative PCR.

Anticipated Results

- If, upon activation of k-ras or Wnt, **only DC or AC led to PDA or SPT formation**, respectively, that would strongly indicate that cellular origin is an important determinant of tumor phenotype.

- If **both DC and AC can form PDA or SPT**, that would indicate tumor phenotype is determined at the genetic level and that both cell types can form a given tumor.

-Finally, if **k-ras or Wnt activation do not form tumors in either DC or AC** (or form tumors that are neither PDA nor SPT), this would suggest 1) PDA or SPT do not arise from AC or DC, or 2) neither cellular origin nor genetic mutations are sufficient to drive tumor formation. I will discriminate between these possibilities by using intraperitoneal caerulein injection to induce pancreatitis because previous studies have shown that PDA form from AC in response to k-ras^{G12V}, but only with pancreatic injury [4].

B. Background and Significance

The pancreas is an essential organ whose main purposes are to control glucose homeostasis and produce digestive enzymes. The organ is comprised of the endocrine and exocrine compartments. α cells and β cells of the endocrine compartment produce glucagon and insulin, respectively, whose opposing actions help regulate blood glucose levels. The exocrine compartment mainly consists of acinar cells that produce digestive enzymes and epithelial cells that line the ducts that deliver the enzymes to the small intestine.

There is a wide spectrum of pancreatic tumors that differ widely in their prognoses. The most common type of pancreatic cancer by far, is PDA, which also is the most lethal form of pancreatic tumors. The cellular origin of pancreatic tumors remains a vital topic for research to further our understanding of carcinogenesis and is the subject of study for this proposal. One of my aims is to determine which pancreatic cell type gives rise to PanIN and PDA. A common strategy used in past studies has been to create transgenic mice carrying conditionally expressed mutations (such as k-ras^{G12D}) that are activated by Cre recombination-excision of a stop signal [2, 4, 5]. These mice are crossed to transgenic mice carrying a Cre gene driven by tissue-specific promoters. The resultant double-transgenic offspring have mutations targeted specifically to a given compartment of the pancreas. However, previous attempts to identify the pancreatic tumor progenitor cells with this strategy have been limited by various factors. One problem is that there are currently no promoters that can target adult pancreatic epithelium. My proposal bypasses this obstacle by first isolating and culturing ductal cells that are then transformed *in vitro* with k-ras^{G12D}/Trp53^{R172H} or β -cat^{ex3} mutations. These cells are then reimplanted into nude mice for observation for tumor development.

Another limitation has been the lack of temporal control over the effects of tumorigenic mutations. For example, even though Grippo et al were able to target k-ras^{G12D} to acinar cells via elastase-driven Cre expression [5], this mutation was carried through all phases of development, starting when the elastase gene was first activated at E13 [9]. I will study the effects of k-ras and β -catenin mutations that occur only in adult mice pancreata, which more closely mimics human disease. For Specific Aim 1, I will use a version of Cre that has an attached estrogen receptor motif so that recombinase activity is induced only in the presence of tamoxifen, which will be introduced only to adult mice. In Specific Aim 2, I will isolate duct cells from adult mice. Notably, Guerra et al recently studied the effects of k-ras mutation targeted to adult mice acinar cells via the elastase promoter in combination with the tet-off strategy and found PanIN/PDA development only in the setting of pancreas injury [4]. However, they used a different type of k-ras mutation (k-ras^{G12V}) than the one that is most often seen in human pancreatic cancer (k-ras^{G12D}), which is the version that we will be studying.

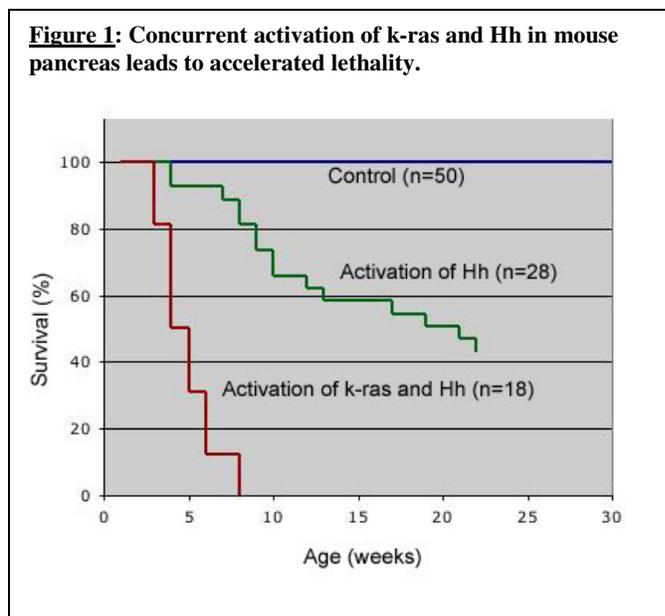
The second aim of my proposal is to determine which cell type gives rise to SPT, a type of pancreatic tumor that has yet to be studied in detail. As will be discussed in Section G, we discovered a novel model for SPT in our studies of the Hedgehog and Wnt pathways in pancreatic tumors. SPT developed

from activating mutation in β -catenin and appear to arise from ductal cells. The work proposed in the Specific Aim 2 will provide new information on origins of SPT. I will also use our SPT model as another tool to test my hypothesis that the cellular origin of tumor is an important factor in determining pancreatic tumor phenotype.

Pancreatic cancer is the fourth-leading cause of cancer deaths in the United States. With 33,000 new cases every year and a five-year survival of 5%, there is a desperate need for improved treatments [10]. To develop more effective therapies, we need a fuller understanding of the mechanisms that lead to tumorigenesis, including whether the cellular origin of pancreatic tumors is important in determining tumor phenotype. My proposal seeks to bridge that gap in our knowledge of pancreatic carcinogenesis. Identifying the types of cells that give rise to pancreatic tumors has several important implications. First, we will be able to focus additional research efforts towards that particular cell type and pursue more detailed studies into the mechanisms behind pancreatic tumorigenesis. Secondly, we may be able to deliver more targeted therapy that has increased efficacy and reduced side effects.

C. Preliminary Studies

Both the Hedgehog (Hh) and Wnt signaling pathways have long been known to be important components in embryonic development, but the exact nature of their contributions to cancer development remains obscure. Previous immunohistochemistry studies on human cancer samples have implicated both pathways in carcinogenesis [11]. Our laboratory found that Hh was abnormally expressed in human PDA even though it is not normally seen in adult pancreata [12]. When we activated Hh in murine pancreatic epithelium we found these mice developed only undifferentiated pancreatic tumors. Based on studies that showed Ras signaling activated the Hh pathway during the formation of pancreatic tumors, we concurrently activated k-ras and Hh in mouse pancreata and found increased PanIN formation and accelerated lethality [13] (Figure 1). This suggests that perturbation of the Hh pathway is sufficient to cause pancreatic neoplasia, but does not lead to PDA, unless there is also k-ras deregulation [13].



As a downstream target of Hh signaling, Wnt has been linked to various carcinomas, including 30-65% of PDAC [14]. We recently discovered that Wnt activation is found in both human PDA and mouse models of PDA and that Wnt activation is regulated by Hh. We also found inhibiting Wnt signaling in PDA cell lines reduced proliferation and increased apoptosis (Figure 2) [6]. This set of observations involving Hh and Wnt suggests a step-wise relationship from k-ras activation to PDA formation, via Hh and Wnt signaling (Figure 3).

On the basis of these findings, we hypothesized that Wnt activation should produce PanIN-PDA. To test this hypothesis, we used an activated allele of β -catenin, which is a downstream effector of Wnt. After Cre-mediated recombination-excision of the third exon of β -catenin (β -cat^{ex3}), the protein product is stabilized from the usual degradation process and leads to constitutive downstream activity in the Wnt pathway [15]. Using Pdx-Cre mice, we expressed β -cat^{ex3} mainly to the acinar compartment, with

mosaic expression in islet cells, and no expression in duct epithelium. We found that despite pancreatic hyperplasia, gross morphology was maintained and no tumor formed [7]. However, when we used the Ptf1a promoter to drive recombination to take place also in the ductal compartment, large pancreatic tumors formed that resembled solid pseudopapillary tumors (SPT), a rare and indolent type of pancreatic neoplasm that harbors activating mutations of β -catenin in up 90% of human samples examined [8] (Figure 4, Table 1). The different lesions produced by the Pdx (acinar, islet) and Ptf1a (acinar, islet, duct) promoters suggest that SPT arise from duct cells.

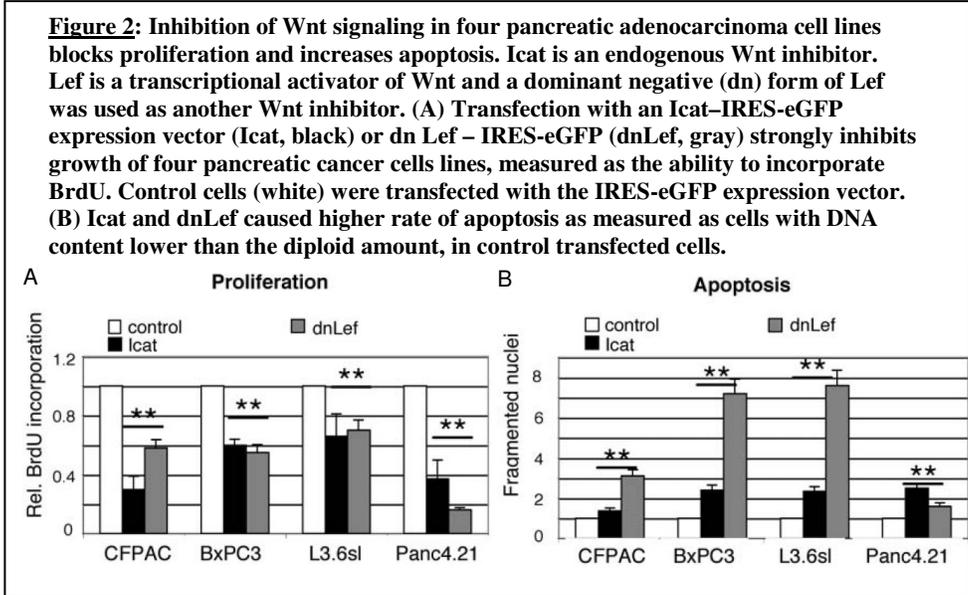
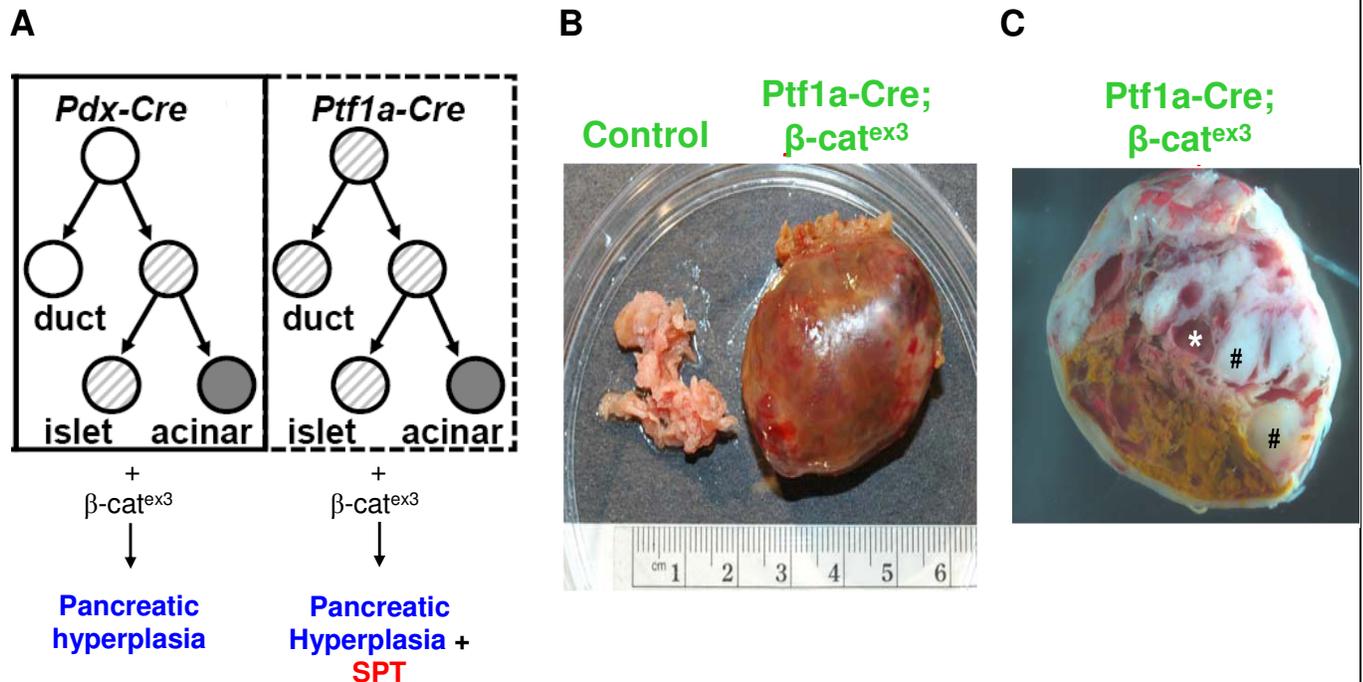


Figure 3: Proposed pathway from k-ras activation (k-ras^{G12D}) to PanIN and PDA formation

k-ras^{G12D} → ↑ Hh → ↑ Wnt → PanIN → PDA

Even though our early work suggested that Hh and Wnt act as intermediaries in a k-ras-PanIN/PDA model, our recent studies showing that Wnt activation leads to SPT formation suggest a more

Figure 4: Pancreatic tumors from mice with activating β -catenin mutation expressed with Ptf1a promoter. (A) Schematic representation of the cellular compartments targeted by the different pancreatic mouse Cre strains, and their respective phenotype when crossed to the β -cat^{ex3} mouse. White circles indicate no Cre expression. Gray striped circles indicate mosaic Cre expression. Dark gray circles indicate robust Cre expression. (B) Gross morphology of tumor seen in 9 month old mouse (right) compared to normal pancreas from a littermate control (left). (C) Gross morphology of a cross section of the murine tumor reveals pseudopapillary regions (labeled by #) and cystic structures (labeled by *).



complicated relationship beyond the simple stepwise progression denoted in Figure 2. This may be due to dissimilar tumorigenic effects that these mutations have when expressed in different cell types.

My proposal will test the hypothesis that cellular origin is an important factor in determining pancreatic tumor phenotype. It is likely that there are different intrinsic properties in acinar and ductal cells, along with their progenitor cells, that allow for diverse transformative responses to given genetic alterations. To test this hypothesis, I will target mutations of k-ras and Trp53 or β -catenin to only ductal or acinar cells and observe for tumor formation. My project will help elucidate the fundamental mechanisms that drive pancreatic tumorigenesis. These results will focus future research in pancreatic carcinogenesis and may lead to desperately needed novel and targeted therapies to treat this deadly disease.

Table 1: Tumors in mice with activating mutation in β-catenin have immunohistological findings that are similar to human solid pseudopapillary tumors.		
	Human SPN	Mouse Tumor
β-catenin	Nuclear/cytoplasmic	Nuclear/cytoplasmic
Synaptophysin	Negative	Negative
α₁-antitrypsin	Focal with globular pattern	Focal with globular pattern
AE1/AE3	Negative	Negative
Chromogranin	Negative	Negative
NSE	Positive	Focal weakly positive
ER	Negative	Negative
PR	60% positive	n/a
Cyclin D1	Positive	Positive

D. Research Design and Methods

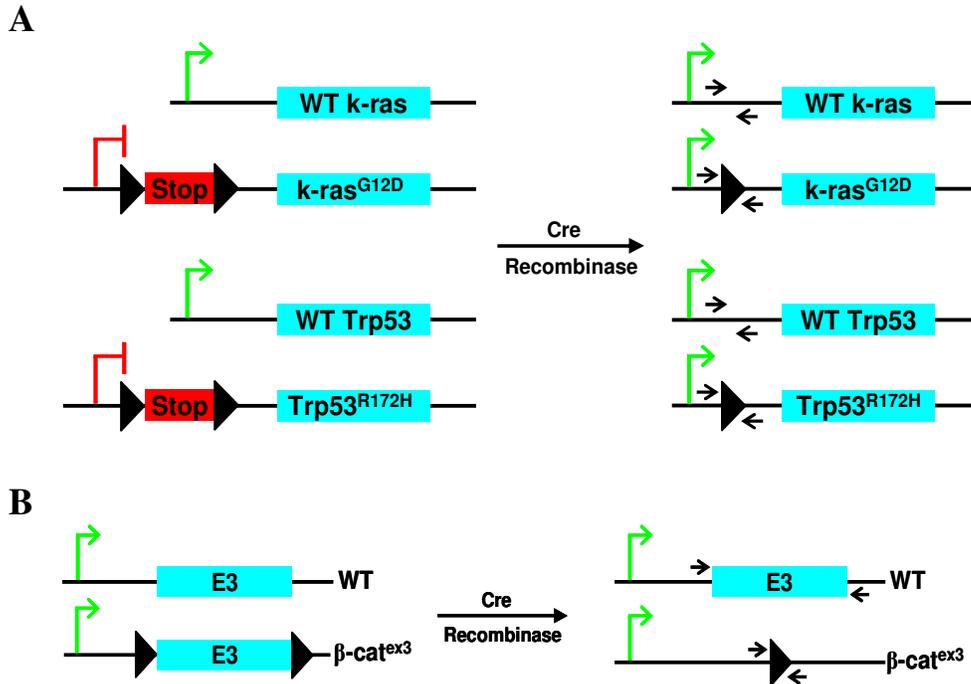
Specific Aim 1: To determine the role of adult pancreatic acinar cells (AC) in the development of pancreatic ductal adenocarcinomas (PDA) and solid pseudopapillary tumors (SPT).

We will breed transgenic mice that have conditionally expressed alleles of k-ras^{G12D}/Trp53^{R172H} and β -cat^{ex3}. The k-ras^{G12D}/Trp53^{R172H} mice are heterozygous for a targeted mutation in the first exon of the k-ras allele and point mutation of Trp53 [2]. The k-ras allele is transcriptionally silent until activated by Cre-mediated recombination excision of a loxP-stop-loxP construct that results in glycine→aspartic acid substitution. This is a mutation commonly found in human PDA that compromises wild-type Ras GTPase activity and leads to constitutive downstream signaling. Trp53^{R172H} is the mouse ortholog of the human mutation that results in Li-Fraumeni syndrome and is the most common p53 mutation found in human PDA [16]. The mutation is an arginine→histidine substitution that is activated when the stop signal is excised. The β -cat^{ex3} model is based on Cre-mediated recombination-excision of the third exon of the β -catenin gene. The mutant protein product lacks the necessary N-terminal phosphorylation sites necessary for ubiquitylation-dependent degradation and is thus stabilized for constitutive downstream activity [15] (Figure 5).

To target the given mutations specifically to adult pancreatic acinar cells, I will use transgenic mice expressing tamoxifen-dependent Cre recombinase under the control of a promoter derived from the elastase gene (Ela-CreER) [9]. The elastase promoter confers tissue specificity while the estrogen receptor fused to Cre allows for recombinase activity only in the presence of tamoxifen, which will be introduced via intraperitoneal injections in adult mice. The estrogen receptor motif provides temporal control over the effects of the given mutation, thus bypassing the consequences of activated k-ras, Trp53, and β -catenin on developing pancreata.

PCR will be used to confirm the excision of the silencing cassette in pancreatic tissues. DNA from other tissues will be used as a control to confirm tissue-specific excision-recombination. Western blotting will

Figure 5: Transgenic mouse models of PDAC and SPT to be used in this proposal. (A) Specific Aim 1: Double transgenic mouse model of pancreatic ductal adenocarcinoma based on mutations in k-ras ($k\text{-ras}^{\text{G12D}}$) and Trp53 ($\text{Trp53}^{\text{R172H}}$) that are activated once Cre-driven recombination excision of the lox-stop-lox silencing cassette takes place. (B) Specific Aim 2: Mouse model of solid pseudopapillary tumors based on activating mutation in β -catenin ($\beta\text{-cat}^{\text{ex3}}$) as a result of Cre-driven recombination-excision of exon 3 that leads to a stabilized protein product that is protected from the normal degradation processes.



be used to confirm the presence of phospho-ERK (a marker for k-ras activation) while expression of stabilized β -catenin will be confirmed via immunohistochemical staining as previously shown [17]. Immunohistochemistry against acinar cell antigens will verify cell-specific expression. In previous experiments using different promoters to drive Cre expression, SPT were first detectable at three months of age [7] and significant PDA were seen by 10 weeks of age [2]. We will inject tamoxifen at 1 month of age and evaluate tumor development at 1, 3, and 6 months after injection. Tumor burden will be quantified and histologic analysis performed to evaluate for phenotype. Tissue fixation and preparation for immunohistochemical studies and Western blotting will be performed as previously described [7]. Immunohistochemical markers for SPT are listed in Table 1 of Section G. Gene expression analysis of the tumors will be performed via quantitative PCR.

Specific Aim 2: To determine the role of adult pancreatic ductal cells (DC) in the development of PDA and SPT.

Because there is currently no promoter that allows for specific gene expression into DC *in situ*, I will adopt a different strategy than the one employed in Specific Aim 1A. I will isolate DC from transgenic mice expressing conditionally expressed $k\text{-ras}^{\text{G12D}}/\text{Trp53}^{\text{R172H}}$ or $\beta\text{-cat}^{\text{ex3}}$ as previously described [18]. Immunohistochemistry against duct cell antigens including mucin-1 and lectin *Dolichos biflorus* agglutinin, will verify the success of the isolation procedure. The cells will be cultured and infected with lentivirus carrying Cre recombinase and luciferase. Recombination will be confirmed with PCR and DNA from other tissues and cells infected with lentivirus without Cre will serve as controls. Western blotting will be used to confirm the presence of β -catenin or phospho-ERK. The luciferase construct includes a red fluorescent protein (RFP) marker, and successful infection can be determined with

Western blotting against either luciferase or RFP. These cells will be re-implanted into nude mice as previously described and observed for tumor growth [12].

Luciferin will be injected intraperitoneally every month. The luciferin allows me to observe transformed ductal cells in real-time with an IVIS Lumina imaging system (Xenogen, Cranbury, NJ). Thus, I can evaluate for metastasis *in vivo* as a marker for malignancy without having to sacrifice the animal. At 1, 3, and 6 months after tumor implantation, the mice will be euthanized and the pancreata harvested and evaluated for tumor development. Sacrifice may occur at earlier time points if there is evidence of metastasis. Tumor burden will be quantified and histologic analysis performed to evaluate for phenotype. Gene expression analysis of the tumors will be performed via quantitative PCR.

Anticipated Problems

Our laboratory has all of the transgenic mice that are needed for the above experiments, except for the Elastase-CreER line, which we are in the midst of obtaining from Dr. Doris Stoffers (Dept. of Medicine, University of Pennsylvania). We have extensive experience isolating pancreatic ductal cells and working with lentivirus. Furthermore, we have on-going collaborations with Dr. David Klimstra (Dept. of Pathology, Memorial Sloan-Kettering Cancer Center) and Dr. Grace Kim (Dept. of Pathology, UCSF) to take advantage of their experience with evaluating pancreatic tumors based on histologic features. All necessary equipment, including the Xenogen imaging system, is readily available on-site. I do not anticipate any other technical problems.

Timetable for Project

SA 1A: Breed k-ras^{G12D}/Trp53^{R172H} x Ela-CreER; β -cat^{ex3} x Ela-CreER

SA 1B: Inject tamoxifen in adult mice

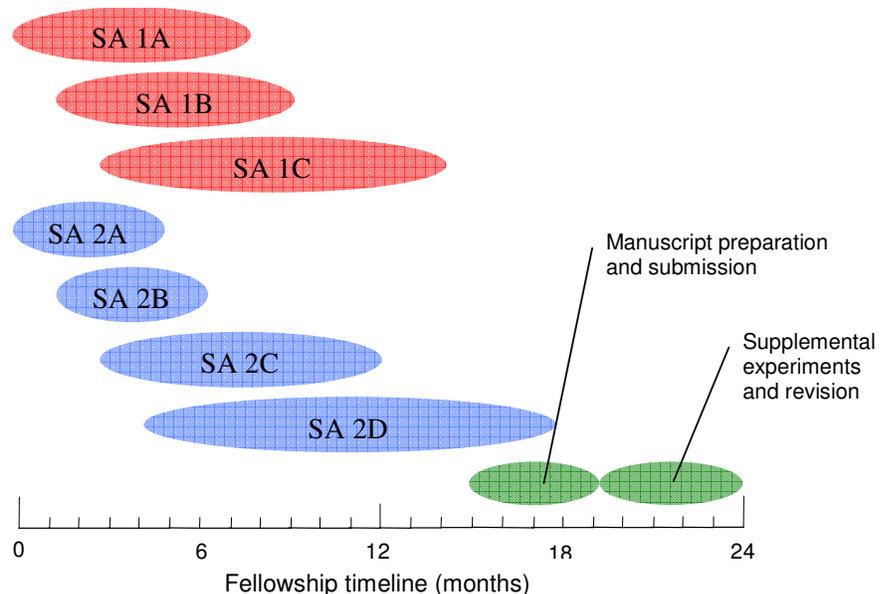
SA 1C: Observe mice for tumor development and characterize lesions

SA 2A: Isolate duct cells from k-ras^{G12D}/Trp53^{R172H} & β -cat^{ex3} mice

SA 2B: Introduce Cre recombinase and luciferase to cultured cells via lentivirus

SA 2C: Re-implant transformed cells into nude mice and monitor for tumorigenesis

SA 2D: Observe mice for tumor development and characterize lesion phenotype



E. Human Subjects Research

No human subjects will be used in this project.

F. Vertebrate Animal

1. Proposed use of the animals: The proposal is based partly on animal studies and mice are the only species used for my experiments. I will use established transgenic lines to determine the role of pancreatic ductal and acinar cells in tumorigenesis.

For Specific Aim 1, I estimate that I will need five mice per injection group for evaluation. The pancreata in β -cat^{ex3} mice are heterozygous for that mutation and in previous experiments developed SPT at about 50% penetrance so only 25% of Ela-CreER x β -cat^{ex3} offspring will develop SPT. Given this rate of expression and three injection groups at five animals per group, I will need approximately 60 animals to study SPT. In past studies, k-ras^{G12D}/Trp53^{R172H} mice are heterozygous for both genes and develop PanIN/PDA at 100% penetrance [2]. Thus, with three injection groups, I estimate that I will need 60 animals for the PDA arm. However, the final number will vary based on penetrance with Ela-CreER promoter.

For Specific Aim 2, I estimate that I will need five samples per time point. Each sample will require two animals (one transgenic donor mouse and one recipient nude mouse). With three time points for two arms, I will need a minimum of 60 animals. The final number will depend on the effectiveness of lentiviral-delivered Cre recombination *in vitro*.

Species	Strain	Ages	Sex	Numbers
Mice	57B6, CD1, FVBN, CBA	Adult	Male and Female	~ 180

2. Choice of species: Mice are the most appropriate species because the methods of homologous recombination and the generation of transgenic animals allow ectopic activation of the target genes (k-ras^{G12D}, Trp53^{R172H}, and β -cat^{ex3}). The murine models of pancreatic ductal adenocarcinoma recapitulate human disease and the results from my proposal will be useful in guiding future research and may lead to the development of novel therapies. We are not aware of any other mouse model of solid pseudopapillary tumor and results from my study will further our understanding of the pathogenesis behind this disease.

3. Housing and veterinary care: The mice are housed in the UCSF barrier facility at Parnassus and are under the care of the Laboratory Animal Resource Center. There is daily observation and care by trained husbandry technicians, and medical care is provided as needed by veterinary technicians and veterinarians. Frequent testing for pathogens is maintained through the Sentinel animal program. Testing for murine pathogens is performed every 10 weeks. Immunocompetent young adult sentinel animals are used to indirectly sample the populations of experimental rodents. The Sentinel program consists of one sentinel animal per rack. The sentinel rodents are exposed to soiled bedding collected from all cages on the rack. The period of exposure is 5 or more weeks after which the sentinel rodents are bled and serum samples are sent to an appropriate testing facility, including the University of California-Davis Comparative Pathology Laboratory and the University of Missouri Research Animal Diagnostic and Investigative Laboratory (MU RADL).

4. Procedures to ensure minimal discomfort and stress: Mice that might develop pancreatic tumors will be monitored for their body condition. Body condition will be evaluated according to the guidelines

described in ‘Body condition scoring: a rapid and accurate method for assessing health status in mice. 1999, Laboratory Animal Sciences, 49’. As it is the case with other solid organ tumors, the early stages of pancreatic tumor will not be evident. I anticipate that a growing tumor will cause the mice to become underconditioned (BC2) and eventually emaciated (BC1). While I anticipate that formation of pancreatic cancer will result in dramatic changes of body condition, the exact characteristics are unknown. Animals will be euthanized when body condition changes dramatically towards stage BC1. The criteria for euthanasia will be: vertebrae is distinctly segmented, skeletal structure is prominent, or significant reduction of flesh cover. These criteria will be posted in the rooms the animals are housed. Mice will be euthanized between BC2 and BC1 before the vertebrae becomes distinctly segmented.

5. *Method of Euthanasia*: Euthanasia is performed according to methods described in the ‘2000 report of the American Veterinary Medical Association (AVMA) panel on euthanasia’. I will use Phenobarbital for general anesthesia for removal of pancreata and tumors from adult animals. Effective anesthesia will be verified by foot pad pinch. The animals will be euthanized by producing bilateral pneumothoraces immediately after the organs and tumors are removed.

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H. Resource Sharing

No model organisms will be developed in this project.

I. Respective Contributions

Over the past year, I have worked closely with my sponsor Dr. Hebrok to develop a research plan that is appropriate for a two-year research period starting in July, 2008. We have had multiple discussions in regards to the direction of the work in his laboratory as it pertains to my interests in pancreatic cancer development and biology. Dr. Hebrok and I developed the research plan presented in the current proposal based on recent work by two postdoctoral fellows in Dr. Hebrok's laboratory - Dr. Pasca di Magliano [6] and Dr. Heiser [7]. I am the sole author of this research training plan, which was produced under the supervision of Dr. Hebrok who has reviewed and guided the direction of this proposal. I also elicited the help of Pamela Derish, who is a scientific editor employed by the UCSF Department of Surgery. She provided helpful suggestions in the overall composition of the research proposal.

J. Selection of Sponsor and Institution

As a general surgery resident training at UCSF, I am acutely aware of the quality of scientific research being performed and the resources available to trainees at our medical center. UCSF provides an excellent training environment for basic science research.

My clinical interest lies in surgical oncology with an emphasis hepatobiliary and pancreatic disease. Based on my interest in investigating the pathogenesis in pancreatic cancer, I approached Dr. Hebrok in regards in training in his laboratory. I was impressed with the exciting progress that he and his colleagues have made in delineating the signal pathways that drive pancreatic tumorigenesis. Dr. Hebrok's research is a blend of basic and applied sciences that allows me to take advantage of my clinical training and experience while providing challenging educational opportunities in the approach to basic science research.

The interdisciplinary nature of Dr. Hebrok's laboratory is another advantage afforded to his trainees. Internally, the Hebrok group studies both pancreas development and pancreatic cancer. Because of the interrelated nature of cancer biology and developmental pathways, the Hebrok lab provides a fertile ground for future breakthroughs in pancreatic carcinogenesis. Externally, Dr. Hebrok's group is part of the UCSF Diabetes Center, which is a collaboration between multiple research groups studying varying aspects of pancreas development and pathology. The Hebrok laboratory is also part of the UCSF Pancreatic Cancer group. The interdisciplinary nature of his laboratory will provide an exciting and stimulating environment to learn from other researchers at UCSF.

K. Conduct of Research

As part of the research training program, the UCSF requires all researchers to participate in a number of training sessions to ensure responsible research conducts. Required classes include:

1. Scientific Ethics. The UCSF Department of Surgery Research Committee holds a mandatory seminar on the responsible conduct of research. This course will occur in September, 2008. Topics will include conflict of interest, ethics of authorship, data management, and issues about animal and human experimentation. These seminars are small group in nature led by various faculty members. This class is limited to UCSF surgery residents at the beginning of their research time and the class size will be approximately seven to 10 residents. The class has five one-hour sessions.
2. Basic Regulatory and Ethical Requirements (BRER). For all UCSF researchers who use animal models, there is required training in the ethics of animal research sponsored by the IACUC designed to meet the requirements of the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act. This online training module will discuss basic regulatory and ethical requirements along with the proper handling, anesthetic, and euthanization protocols.
3. Mouse Basic Class: All UCSF researchers who use mice models are required to attend this class to learn the assessment of animal health, handling and restraint techniques, injection techniques, common anesthetic agents, assessment of the depth of anesthesia, euthanasia, and safety, health and environmental considerations. This is a small-group hands-on training session. It is a one-time three hour course.