Defining the Contributions of Pancreatic Ductal and Acinar Cells to Tumorigenesis

Training Objectives:

I plan to spend both years of the fellowship working full-time as a research associate in the laboratory of my mentor, Dr. Matthias Hebrok. This experience will train me to evaluate basic science literature, form hypotheses, and plan experiments utilizing the latest techniques. These are essential skills for me to learn as I plan to pursue a career in academic surgery with an emphasis in basic science research. The subject of my proposed project in pancreatic carcinogenesis will allow me to pursue my interest in oncology.

Research Proposal

A. Specific Aims

One of the fundamental topics in pancreatic cancer research is the cellular origin of tumorigenesis. The pancreas is composed of many types of exocrine and endocrine cells. Mirroring the diversity of cell types, there is a wide-range of pancreatic tumors that have traits reflecting characteristics of normal cells that suggest at the source of tumorigenesis. For example, because pancreatic ductal adenocarcinomas (PDAC) resemble normal ductal cells histologically, it is natural to assume that ductal epithelium is the site of origin. However, studies have suggested that other cell types may be the source of PDAC. Our laboratory has recently developed mouse models of several different types of pancreatic tumors. We found that triggering the Wnt pathway resulted in solid pseudopapillary tumor (SPT) formation, while concurrent activation of k-ras and Wnt led to acinar cell carcinoma-like (ACC) lesions. The latter finding is surprising as activation of k-ras signaling in the absence of exogenous Wnt activation results in PDAC. Here, I propose to use these pancreatic tumor mouse models to define the contributions of pancreas acinar and duct cells to PDAC, SPT, and ACC formation.

Specific Aim 1: To determine the role of acinar cells (AC) in the development of pancreatic ductal adenocarcinomas (PDAC), solid pseudopapillary tumors (SPT), and acinar cell carcinoma-like lesions (ACC).

We will breed transgenic mice that express conditionally activated k-ras ($k\text{-}ras^{G12D}$) and/or β-catenin ($\beta\text{-}cat^{ex3}$) specifically in pancreatic AC (via an Elastase promoter). By localizing these mutations to adult AC, we will monitor for tumor development when only that cell population has been genetically altered.

Specific Aim 2: To determine the role of pancreatic ductal cells (PDC) in the development of PDAC, SPT, and ACC.

Because there is currently no promoter that allows for specific gene expression into PDC in situ we will adopt a different strategy to evaluate the role of pancreatic duct epithelium in tumorigenesis. We will isolate and culture ductal cells from $k\text{-}ras^{G12D}$, $\beta\text{-}cat^{ex3}$, and $k\text{-}ras^{G12D}/\beta\text{-}cat^{ex3}$ mice. Cre recombinase will be introduced via lentivirus to activate the given mutations. These cells will be re-implanted into nude mice and monitored for tumorigenesis. A luciferase gene will also be introduced at the same time as Cre so that we can use in vivo, real-time luminescence to evaluate for metastasis.

B. Background and Significance

Pancreatic cancer is the fourth-leading cause of cancer deaths in the United States. The incidence is approximately 33,000 new cases per year that roughly matches the number of annual deaths. The 5% five-year survival rate speaks to the obvious need for improved treatments [1].

Recently, there has been a surge in the understanding of molecular mechanisms that underlie pancreatic tumorigenesis. Currently, more than 90% of newly diagnosed pancreatic

cancers are ductal adenocarcinomas (PDAC). The majority of these lesions originate from low-grade precursor tumors termed pancreatic intraepithelial neoplasms (PanIN). K-ras activation is found in most human pancreatic cancers and is one of the earliest genetic alterations detected in PanIN [2]. In mice, mutational activation of k-ras (k-ras^{G12D}) recapitulates the human disease process [3]. Recently, other tumor suppressors and oncogenes have been implicated in the development of a wide range of pancreatic neoplasms [4].

The cellular origin of pancreatic tumors is a source of active interest. The pancreas is composed of acinar, endocrine, and ductal cells. In terms of PDAC, because these tumors have histological resemblance to normal ductal epithelium, it is assumed that duct cells are the source of carcinogenesis. However, there are other possibilities. Acinar-to-ductal metaplasia is often associated with malignancy, thus raising the possibility that acinar cells may be the source of tumorigenesis [4]. Indeed, a recent study showed k-ras activation that is restricted to pancreatic embryonic cells of acinar and centroacinar lineage led to PanIN and PDAC formation [5]. Establishing the source of tumorigenesis is a fundamental and unresolved issue in pancreatic cancer research. The cells of origin may solely determine pancreatic tumor phenotype. Alternatively, it may be the unique combination of genetic "hits" amassed by pancreatic cells, rather than the cells of origin, that determines tumor phenotype. It is the goal my proposal to distinguish between these possibilities.

C. Preliminary Data

Both Hedgehog (Hh) and Wnt have long been known to play an important role in embryonic development, but the exact nature of their contributions to cancer development remains obscure. Previous histological studies on human tumor samples and recent work from our laboratory have implicated the Hh and Wnt signaling pathways in pancreatic tumorigenesis [6, 7]. Based on this work, we have developed mouse models of several pancreatic tumors.

In PanIN-PDAC lesions, we found that k-ras activation led to Hh signaling, that in turn activated the Wnt pathway. These results imply a step-wise relationship from k-ras activation to PDAC formation, via Hh and Wnt signaling (Figure 1) [8]. This model suggests that Hh or Wnt activation would also produce PanIN-PDAC. While simultaneously activating k-ras and Hh (via GLI2, a downstream mediator of Hh) resulted in PanIN-PDC, triggering Hh alone led to only undifferentiated tumor formation [9] (Table 1).

Figure 1: Exogenous k-ras activation triggers Hh and Wnt and leads to PanIN and PDAC formation

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

When we perturbed the Wnt pathway, the results were also confounding. Triggering Wnt alone via an activating mutation of β-catenin (β-cat^{ex3}), which is the downstream effector in the Wnt pathway, led to formation of solid pseudopapillary tumors (SPT), a rare and indolent type of pancreatic neoplasm, without evidence of PanIN-PDAC. More interestingly, when k-ras and Wnt were activated together, acinar cell carcinoma-like tumors (ACC) formed without PanIN-PDAC (unpublished data, Table 1). Like SPT, ACC is rare and comprises less than 1% of pancreatic tumors. However, it is more malignant in nature. This finding is notable because k-ras activation in the absence of exogenous Wnt activation results in PDAC formation.

Table 1: Mouse models of pancreatic neoplasms

Gene	Tumor Phenotype
k-ras^{G12D}	PanIN - PDAC
GLI2 (Hh activation)	Undifferentiated tumors
k-ras^{G12D}/GLI2	PanIN - PDAC
β-cat^{ex3} (Wnt activation)	SPT
k-ras^{G12D}/β-cat^{ex3}	ACC-like tumor

Even though our early work suggested that Hh and Wnt act as intermediaries in a k-ras-PanIN-PDAC progression model, our recent studies suggest a more complicated relationship.

Currently, it is unknown whether each type of pancreatic tumor arises from a unique cell type that is transformed when certain signaling pathways are perturbed. Alternatively, the tumors may originate from the same cells but the phenotype is determined by the combination of genetic changes. The objective of the proposed study is to establish the role that each pancreatic cell type plays in the formation of various pancreatic tumors.

D. Research Design and Methods

Specific Aim 1: To determine the role of acinar cells (AC) in the development of pancreatic ductal adenocarcinomas (PDAC), solid pseudopapillary tumors (SPT), and acinar cell carcinomas-like lesions (ACC).

We will breed transgenic mice that have floxed k-ras^{G12D}, β-cat^{ex3}, or k-ras^{G12D}/β-cat^{ex3} (double transgenic). These mice will be crossed with the Elastase-CreER mice [10]. The elastase promoter directs Cre recombinase expression exclusively to adult pancreatic acinar cells so that the given mutations will occur in a tissue-specific manner. The estrogen receptor fused to Cre induces recombinase activity in the presence of tamoxifen, which will be introduced via intraperitoneal injections in adult mice. PCR will be used to confirm the excision of the loxP sites. Western blotting and immunohistochemistry will be used to confirm the presence of β-catenin or phospho-ERK, which signifies k-ras activation. At various time-points (4, 8, and 16 weeks) after tamoxifen injection, the mice will be sacrificed and the pancreata harvested and evaluated for tumor development. Tumor burden will be quantified and histologic analysis performed to evaluate for phenotype.

Specific Aim 2: To determine the role of pancreatic ductal cells (PDC) in the development of PDAC, SPT, and ACC.

We will isolate PDC from transgenic mice expressing floxed k-ras^{G12D}, β-cat^{ex3}, or k-ras^{G12D}/β-cat^{ex3}. Each of the three types of cells will be cultured and infected with lentivirus carrying Cre recombinase. Recombination will be confirmed with PCR, Western blotting, and immunohistochemistry. A lentiviral delivery system will also be used to deliver the luciferase gene into each type of cells. The luciferase construct also contains 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 [8]. Via intraperitoneal injection of the luceferin, metastasis may be tracked in vivo and in real-time with a Xenogen imaging system available in the mouse facility at our institution. Tumor burden will be quantified and histologic analysis performed to determine phenotype.

Potential Problems:

Our laboratory has all of the transgenic mice that are needed for the above experiments, except for the Elastase-CreER line. We will obtain a breeding pair from the Melton lab so that the necessary number of mice will be available by the time I start my research period. We have extensive experience isolating pancreatic ductal cells, analyzing pancreatic tumor phenotypes, and working with lentivirus. All necessary equipment, including the Xenogen imaging system, is readily available on-site. As a result, we do not anticipate any technical problems.

E. Summary

Our group has implicated Hh and Wnt as mediators in pancreatic tumorigenesis. However, the cellular origin of various pancreatic neoplasms remains uncertain. The goal of this project is to delineate the contributions that certain pancreatic cell types make to tumorigenesis. Identifying the source of tumor formation should provide the basis for novel therapies.

F. References

1. Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., Smigal, C., and Thun, M.J., *Cancer statistics, 2006.* CA Cancer J Clin, 2006. **56**(2): p. 106-30.
2. Hruban, R.H., Takaori, K., Klimstra, D.S., Adsay, N.V., Albores-Saavedra, J., Biankin, A.V., Biankin, S.A., Compton, C., Fukushima, N., Furukawa, T., Goggins, M., Kato, Y., Kloppel, G., Longnecker, D.S., Luttges, J., Maitra, A., Offerhaus, G.J., Shimizu, M., and Yonezawa, S., *An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms.* Am J Surg Pathol, 2004. **28**(8): p. 977-87.
3. Hingorani, S.R., Petricoin, E.F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M.A., Ross, S., Conrads, T.P., Veenstra, T.D., Hitt, B.A., Kawaguchi, Y., Johann, D., Liotta, L.A., Crawford, H.C., Putt, M.E., Jacks, T., Wright, C.V., Hruban, R.H., Lowy, A.M., and Tuveson, D.A., *Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse.* Cancer Cell, 2003. **4**(6): p. 437-50.
4. Hezel, A.F., Kimmelman, A.C., Stanger, B.Z., Bardeesy, N., and Depinho, R.A., *Genetics and biology of pancreatic ductal adenocarcinoma.* Genes Dev, 2006. **20**(10): p. 1218-49.
5. Guerra, C., Schuhmacher, A.J., Canamero, M., Grippo, P.J., Verdaguer, L., Perez-Gallego, L., Dubus, P., Sandgren, E.P., and Barbacid, M., *Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice.* Cancer Cell, 2007. **11**(3): p. 291-302.
6. Taipale, J. and Beachy, P.A., *The Hedgehog and Wnt signalling pathways in cancer.* Nature, 2001. **411**(6835): p. 349-54.
7. Abraham, S.C., Klimstra, D.S., Wilentz, R.E., Yeo, C.J., Conlon, K., Brennan, M., Cameron, J.L., Wu, T.T., and Hruban, R.H., *Solid-pseudopapillary tumors of the pancreas are genetically distinct from pancreatic ductal adenocarcinomas and almost always harbor beta-catenin mutations.* Am J Pathol, 2002. **160**(4): p. 1361-9.
8. Thayer, S.P., di Magliano, M.P., Heiser, P.W., Nielsen, C.M., Roberts, D.J., Lauwers, G.Y., Qi, Y.P., Gysin, S., Fernandez-del Castillo, C., Yajnik, V., Antoniu, B., McMahon, M., Warshaw, A.L., and Hebrok, M., *Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis.* Nature, 2003. **425**(6960): p. 851-6.
9. Pasca di Magliano, M., Sekine, S., Ermilov, A., Ferris, J., Dlugosz, A.A., and Hebrok, M., *Hedgehog/Ras interactions regulate early stages of pancreatic cancer.* Genes Dev, 2006. **20**(22): p. 3161-73.
10. Murtaugh, L.C., Law, A.C., Dor, Y., and Melton, D.A., *Beta-catenin is essential for pancreatic acinar but not islet development.* Development, 2005. **132**(21): p. 4663-74.