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Insights into the Pathogenesis of Pancreatic Cystic Neoplasms

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Due to increasing availability and use of cross-sectional imaging modalities, the diagnosis of pancreatic cystic neoplasms is on the rise. Over 2% of patients undergoing cross-sectional imaging will be found to have pancreatic cysts [1, 2]. Once discovered, primary care physicians, internists and surgeons are faced with the complex challenge of management of these lesions. Research has shown that ‘some’ of these cysts indeed have malignant potential. Thus, addressing these cysts surgically offers an opportunity to achieve, at least partially, the ever-elusive goal of preventing development of pancreatic cancer. However, not all cysts are pre-malignant and the current modalities used to differentiate the pre-malignant cysts from those which are primarily benign (imaging, endoscopic ultrasound and cyst fluid analysis) while helpful are not completely fool-proof. This often leads to unnecessary surgeries accompanied with their associated complications. For example, up to 20% of pancreatic cysts resected due to concerns about their malignant potential, even at a high-volume center, were found to be benign on final pathology[3]. More importantly the risk of development of cancer in pre-malignant cysts and the factors which drive development of cancer in these pre-malignant cysts are not known. This leads to surgical removal of many pre-malignant cysts, which may have never evolved into pancreatic adenocarcinoma and thus could have been safely observed. A better understanding of the pathogenesis of pancreatic cyst and their progression to malignancy will help make an accurate diagnosis and predict behavior. This review addresses our current knowledge of the pathogenesis of pancreatic cysts.

Classification of Pancreatic Cysts

Pancreatic cysts can be classified into inflammatory and neoplastic subtypes. Inflammatory pancreatic cysts, the most common pancreatic cysts, are typically a local complication of severe pancreatic inflammation. Neoplastic cysts, the focus of the current review, are

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believed to represent 10–15% of cystic lesions [4] and have variable malignant potential. Overall, intra-ductal papillary mucinous neoplasm (IPMN), mucinous cystic neoplasm (MCN) and serous cystadenoma (SCA) are the most common cystic neoplasms of the pancreas. IPMN and MCN are believed to potentially lead to pancreatic ductal adenocarcinoma whereas SCA are largely benign.

Intraductal papillary mucinous neoplasms

Intraductal papillary mucinous neoplasms or IPMN are believed to arise from pancreatic ducts. IPMN are believed to be field defects and may manifest as diffuse or multi-focal involvement. The epithelial lining of the IPMNs demonstrate a spectrum of dysplasia ranging from low to high grade progressing to invasive cancer. Based on the site of origin, main pancreatic duct, branch ducts or the mixture of both, these neoplasms have been classified into main duct IPMN (MD-IPMN), branch duct IPMN (BD-IPMN) or mixed type, where both main duct and branch duct are involved, respectively. This classification is not only of academic interest but actually helps predicts biology. The involvement of main duct, which is evident on the preoperative cross-sectional imaging, suggests more aggressive biology and up to 60% of resected MD-IPMN will demonstrate carcinoma-in-situ and/or invasive cancer[4]. On the other hand, malignancy is reported in 12–30% of resected BD-IPMN[4]. If considered as a whole, malignancy risk may be even lower in BD-IPMN, as many of these are observed and only the most concerning are resected.

Detailed histopathological analysis has helped classify the IPMNs into gastric, intestinal, pancreatobiliary and oncocytic papillary subtypes. Interestingly, this histopathological classification also co-relates with biology. Epithelial morphology in gastric subtype resembles gastric mucosa and is characterized by a low proliferative activity and consequently a lower risk of malignant transformation. This subtype is most commonly associated with BD-IPMN. Intestinal subtype, as the name suggests, recapitulates the epithelium of colonic villous adenomas. Many of the MD-IPMN bear this morphology and invasive disease developing in this subtype gives rise to the colloid variety of PDAC. The most sinister pattern, however, is the pancreatobiliary subtype, which typically harbors high grade dysplasia with invasive lesions developing to form tubular PDAC.

Mucinous cystic neoplasm

Mucinous cystic neoplasms or MCN are seen almost exclusively in females, are almost always solitary and may be located in either the body or the tail of the pancreas. In contrast to IPMN, MCNs tend to be solitary and do not communicate with the pancreatic duct. The epithelial lining may exhibit varying degree of atypia and based on the this the cysts are classified into 1) mucinous cystadenoma; 2) mucinous cystic tumors; and 3) mucinous cystadenocarcinoma. One of the unique features of MCNs is the dense cellular ovarian type stroma which underlines the epithelium. This unique stroma may help distinguish MCNs from branch duct IPMN and is considered essential for the diagnosis of MCNs.

Serous Cystadenoma (SCA)

While there are occasional case reports of SCA metastasizing, these lesions are considered benign [4]. However, they can grow to enormous size and may produce local compressive

effects, thus necessitating resection. Macroscopically these lesions are described as microcystic with characteristic septations and thick fibrous wall giving them a honeycomb appearance. These cysts contain thin serous fluid in contrast to IPMN and MCN which have thick mucinous fluid. The cysts are lined by thin glycogen rich cuboidal epithelium. 10–15% of SCAs are oligo- or macrocystic which can make them difficult to distinguish from MCNs or IPMNs[4].

Genetic Makeup of IPMN

While ‘environmental’ factors may initiate and/or drive the neoplastic transformation, genetic alterations are believed to be required for neoplasia. The -omics revolution has helped us decipher and quantitate these genetic changes, thus helping us understand the pathogenesis of various neoplasms. In this regard cystic neoplasms of the pancreas are no exceptions. A variety of studies have evaluated the mutational profile of cystic neoplasms of the pancreas with the goal to get an insight into their pathogenesis. To understand the genetic mutation profile of IPMNs, Wu et al [5] analyzed the sequence of 169 common cancer-related genes in cyst fluid from 19 IPMNs through massive parallel sequencing. The important mutations so discovered were further validated in additional 113 specimens that included cyst fluid and surgically resected specimens of cyst walls. In the initial limited evaluation, it was observed that only 2 genes were mutated in more than one IPMN, namely *KRAS* (14/19 specimens) and *GNAS* (6/19 specimens). All the *KRAS* mutations were at codon 12 (G12D, G12V or G12R) and those in *GNAS* were at codon 201 (R201H or R201C). While *KRAS* mutations have been described in IPMN before, this was the first report of *GNAS* mutation in IPMN. *GNAS* encodes for Gs α which forms a trimer with β and γ subunits and is an integral part of signaling mediated by G-protein-coupled receptors.

GNAS mutation leads to disruption of the intrinsic hydrolytic activity of Gs α , which results in constitutive activation of its function. The frequency of *KRAS* codon 12 and *GNAS* codon 201 mutations was further validated in the validation set of genomic DNA obtained from cyst fluid or macro- and micro-dissected cyst walls. In this large set of IPMNs 66% were found to have *GNAS* mutation and 81% were found to have *KRAS* mutations. Interestingly, 51% of IPMNs had both *KRAS* and *GNAS* mutations, whereas <5% did not have either. No *KRAS* or *GNAS* mutation was observed in serous cystic neoplasms studied in this study suggesting that *KRAS* and/or *GNAS* mutations may be required specifically for the formation of IPMNs. Furthermore, authors observed that there was no correlation between the grade of tumor and the mutation in *GNAS* and *KRAS* genes and that these mutations are also found in IPMNs with no evidence of malignancy. This suggest that these mutations are probably acquired early in the pathogenesis of IPMN and additional mutations may be required for the disease progression. *GNAS* and *KRAS* were also found in 7 out of 8 pancreatic ductal adenocarcinoma developing in the setting of IPMN suggesting that the clones evolving into cancer harbor mutations in these genes. This is in stark contrast with the previously published data on mutations in PDAC, developing in the absence of IPMN, where no *GNAS* mutation was observed [6].

In another small study, Wu et al [7] extended their previous work and used whole exome sequencing on eight IPMNs and matched normal tissues. Authors observed on an average 26

mutations per IPMN. Six genes were mutated in more than one IPMN patient in this small study. Besides reconfirming that *KRAS* and *GNAS* mutations are frequent in IPMNs, this study also demonstrated, for the first time, frequent mutation in *RNF43*. Mutations in *RNF43*, which is known to have intrinsic E3 ubiquitin ligase activity, were even more common than those in either *KRAS* or *GNAS*. *RNF43* mutations were also observed in mucinous cystic neoplasm but not in SCN and SPN. These mutations were the result of both loss of heterozygosity (LOH) as well as base pair substitution.

Previous studies have suggested that besides activating mutations in *KRAS*, IPMN harbor inactivating mutations in many other genes including *CDKN2A/p16* [8], *TP53*, *BRAF*, *PIK3CA* [9] and *SMAD4* [8]. In a targeted next generation sequencing (NGS) based study by Amato et al [10], 52 specimens (48 IPMN's and 4 ITPN's) were studied after enriching for neoplastic cells. Immunohistochemistry (IHC) was used to validate the differential expression of *CDKN2A/p16* and *SMAD4* genes. Confirming previously published findings *GNAS* and *KRAS* mutations were the most prevalent alterations found in 75% and 46% of cases respectively. Taken collectively 87% of cases harbored *GNAS* and/or *KRAS* mutations and only 6 out of 52 samples, which were equally divided between samples with and without associated malignancy, did not have mutations in *KRAS* or *GNAS*. This suggests that, hitherto unknown, alternate early mutations may exist which can initiate the development of IPMNs in a subset of patients. This study also demonstrated that mutations in *RNF43* were third most common mutations and were always associated with mutations in *KRAS* or *GNAS*. Of note, this study did not comment on whether mutation in *RNF43* varied with the grade of the lesion which would have helped elucidate its role in the neoplastic progression of IPMNs. Mutations in the *TP53* and *BRAF* were present in 10% and 6% of the specimens respectively. It is worth noting that the specimens with *TP53* and *BRAF* mutation harbored high grade and/or malignant lesions suggesting that these mutations are acquired later in the pathogenesis of IPMN and might be involved in its progression (Figure 1A). This data corroborates with previous studies suggesting that the frequency of mutations in TP53 gene increases as the lesions progress from adenoma to invasive disease [11, 12].

Mutations in other genes including *CTNNB1*, *IDH1*, *STK11*, *PTEN*, *ATM*, *CDH1*, *CDKN2A*, *FGFR3*, *NRAS*, *SMAD4* were found at a much lower level of less than 4%. The involvement of *CDKN2A/p16* and *SMAD4* in pathogenesis of IPMN was further evaluated by IHC. Intriguingly, *p16* loss on IHC increased with increasing grade of the lesions with 31% of intermediate grade and 53% of high grade lesions demonstrated loss of *p16* and none of the low grade lesions demonstrated *p16* loss (Figure 1A). This data is similar to previous report where loss of *p16* was found to increase as the disease progressed from non-invasive (10% samples with *p16* loss) to invasive disease (100% loss of *p16* in invasive disease) in IPMN [8] (Figure 1A). *SMAD4* loss was observed in four IPMNs associated invasive cancers but the neighboring non-invasive components had intact *SMAD4*. There is some controversy with respect to *SMAD4* loss and IPMN. While an initial study in 79 patients suggested that *SMAD4* is rarely lost even in the invasive cancer secondary to IPMN [13], latter studies have suggested that *SMAD4* does get deleted in IPMN with development of cancer [8].

Besides the mutations and pathways described above, a number of other pathways have been observed to be dysregulated in IPMN. Studies describing these pathways have generally

taken a targeted rather than global approach (like whole exome sequencing). Phosphatidylinositol-3 kinases (PI3Ks) are an important group of lipid kinases which regulate several growth, invasion, and metabolism related signaling pathways. *PIK3CA*, which is a class IA PI3K, has been found to be mutated in several tumor types including gastric, colorectal, lung and breast cancer[14]. Schonleben et al [9] did a mutational profile on 36 IPMN's by direct genomic sequencing and observed that *PIK3CA* was mutated in 11% (4/36) specimens. Possibly due to small number of specimens, it could not be deciphered at what stage of the pathogenesis of IPMN these mutations are acquired.

Do *GNAS* vs *KRAS* mutation predict different biology or developmental pathway

IPMN is an important clinical entity due to the potential of it evolving into malignancy. Invasive cancer developing in IPMN, interestingly, has 2 distinct histopathologic subtypes with different prognoses. Patients with colloid carcinoma developing in patients with IPMN have markedly better prognosis when compared with patients who develop tubular carcinoma [15]. There is also a correlation between histological subtype of the IPMN and the histological subtype of malignancy. Colloid carcinoma typically arise from intestinal subtype of IPMN whereas the tubular carcinoma arises from the pancreatobiliary subtype [16]. Interestingly, studies suggest that mutation profile may drive the specific histopathology of the IPMN as well as the invasive cancer developing in them. Wu et al [5, 7] observed that while *GNAS* and *KRAS* mutations are present in all three histological subtypes of IPMN (intestinal, pancreatobiliary and gastric), *GNAS* mutations predominate in intestinal subtypes and *KRAS* mutations are present in 100% of pancreatobiliary, 83% of gastric but only 39% of intestinal subtype of IPMN. In a study by Tan et al[17], where the mutation profile of IPMN and that of invasive component developing in them was determined by targeted massive parallel sequencing, *GNAS* mutations were more prevalent in colloid carcinoma as compared to tubular subtype (89% vs 52%). *GNAS* mutations were also found to be more prevalent in the intestinal subtype (74%) when compared to pancreatobiliary (31%) and gastric subtypes (50%). The authors did not find any statistically significant correlation between the *KRAS* or *RNF43* mutations and histological subtype of non-invasive IPMN or invasive cancer developing in them. Though the number of patients were small, authors observed a non-significant trend towards improved survival in invasive-IPMN with *GNAS* mutant *KRAS* wildtype when compared to those with *KRAS* mutation. Oncocytic variant of IPMN seem to evolve on a completely different pathway. Basturk et al [18] performed high-depth-targeted next-generation sequencing for a panel of 300 key cancer-associated genes on nine typical pancreatic 'oncocytic subtype' of IPMN. Interestingly, none of the samples had *KRAS* or *GNAS* mutation and only one had *RNF43* and *PIK3R1* mutations suggesting that the oncocytic subtype is not only morphologically but also genetically distinct.

Inflammatory Pathways and IPMN

Recent evidence suggests that progression of pancreatic cyst dysplasia and inflammation may be intricately related. Maker et al [19] performed inflammatory cytokine analysis on cyst fluid recovered from resected IPMNs from 40 patients. The authors observed that IFN- γ and IL-4 were present at very low levels and IL-2, IL-10, IL-12, IL-13 and TNF- α were

present at higher levels. However, none of these cytokines correlated with degree of dysplasia in the specimens. Interestingly, univariate analysis suggested that IL-1 β and IL-5 was present at higher levels in high-risk cysts. IL-1 β levels remained significant predictor of high-risk vs low-risk cysts even after adjusting for IPMN subtype (main duct vs branch duct) and cyst size. At a cutoff point of 1.26pg/ml, IL-1 β levels were 79% sensitive and 95% specific in distinguishing high risk vs low risk cyst. Authors validated these results in a blinded fashion on an additional set of 15 patients and demonstrated that IL-1 β levels of 1.26pg/ml had a positive predictive value of 71% and negative predictive value of 75% to distinguish high risk from low risk lesions. In this study IL-1 β was barely detectable in non-mucinous serous cystadenomas which have a very low if any malignant potential. It must be noted that IL-1 β is a major cytokine that is produced by activated inflammatory cells (primarily monocytes) and has been found to be related to carcinogenesis and chronic inflammation in a number of cancers.

Sadot et al [20] evaluated the association of neutrophil infiltration and malignant progression in IPMN. IPMN lesions were classified as Tumor Associated Neutrophil (TAN) negative (with 10 or fewer neutrophils per 100 tumor cells), low TAN (with 11 to 15 TANs per 100 tumor cells) and high TAN (with more than 15 TANs per 100 tumor cells). All the patients with invasive disease were TAN positive and 90% had high TAN. This data, while not causal, again supports a strong association of inflammation and disease progression in IPMNs. In another study [21] endoscopic ultrasound (EUS) guided fine needle aspirate (FNA) specimens from pancreatic cyst fluids were used to characterize and compare inflammatory cytokines and proteins in inflammatory cysts and BD-IPMNs. In a small set of 5 patients each with either inflammatory cysts or IPMNs, the authors found 7 cytokines to be elevated only in BD-IPMN. These were 6CKine (chemokine with 6 cysteines), CTACK (cutaneous T-cell- attracting chemokine), ENA-78 (epithelial-derived neutrophil-activating protein 78), IL-20, IL-28A, MIP-3a (macrophage inflammatory protein 3-alpha), and MIP-3b. Conversely 11 inflammatory cytokines were elevated exclusively in inflammatory cysts including M-CSF, I-309 (T lymphocyte-secreted protein), IL-17, IL-5, IL-9, TGF- β 1 (transforming growth factor beta-1), TGF- β 2, TGF- β 3, TNF- β , TPO (thyroid peroxidase), and TSLP (thymic stromal lymphopoietin). Of note though, from a total of 89 cytokines assayed, a majority of these cytokines were found to be elevated in both types of lesions suggesting a common inflammatory milieu that may be shared by both inflammatory cysts and pre-neoplastic pancreatic IPMNs. This limited analysis also suggested that granulocyte macrophage colony stimulating factor (GM-CSF) and hepatocyte growth factor (HGF) in EUS-FNA collected pancreatic cyst fluid could distinguish BD-IPMN from inflammatory cysts.

Genetic profiling of Mucinous Cystic Neoplasms

As mentioned above, besides having cysts with varying degree of atypia in epithelial lining, MCNs have a unique ovarian like stroma. Thus understanding of the pathogenesis of MCNs requires an understanding of the development of unique stroma. MCNs harbor many of the same mutations as seen in IPMNs and pancreatic ductal adenocarcinoma with some unique differences. Wu et al [7] demonstrated that in their whole exome analysis of 8 patients with MCN on an average 16 mutations were discovered in each sample, which were more than

SCAs but less than that in IPMNs. Similar to that in IPMNs, *KRAS* mutations were the most common and were observed in 75% of the samples. However, unlike that in IPMNs, *GNAS* mutations were not observed. Both *TP53* and *RNF43* mutations were also observed. Similar to that in IPMNs, *KRAS* mutations are believed to be early event in the pathogenesis of MCNs as even the lesions with low grade dysplasia harbor *KRAS* mutations[22]. On the other hand, *p53* mutations are only observed in malignant lesions thus suggesting that they may have a role in evolution of the invasive component [22, 23]. *SMAD4* or *DPC4* is intact in the non-invasive epithelium of MCN but is lost in the invasive component[24]. Similarly, *PIK3CA* mutations occur in higher grade lesions in MCNs [25]

While mutational profile of the ovarian stroma has not been performed, gene expression analysis of the ovarian stroma of MCN demonstrates overexpression of multiple genes. Stroma of MCNs overexpress several genes involved in estrogen metabolism including *STAR* and *ESR1*[26]. Secreted *Frizzled-related protein* is also overexpressed in ovarian type stroma of MCNs [26]. Wnt signaling has been found to be activated in the stroma of MCNs [27]. In mouse models, overexpression of *Wnt1* in pancreatic acinar cells and acinar cells progenitors leads to development of cystic lesions which resemble MCNs and have Wnt receptor- and progesterone receptor-positive ovarian stroma [27].

Genetic profiling of Serous Cystadenoma

In contrast to IPMN and MCNs, SCAs are considered benign without any malignant potential. Wu et al [7] studied the exome sequence of DNA from the neoplastic epithelium of 8 resected SCAs. They observed that SCAs have 10 ± 4.6 mutations per tumor. Only 2 mutations were present in more than one SCA. Four out of eight SCAs had mutation in von Hippel-Lindau gene (*VHL*). Three of these four demonstrated LOH of the *VHL* chromosomal region. Allelic deletion and mutations in the *VHL* gene has been described previously in SCAs [28]. This is interesting as SCAs are commonly associated with patients with VHL syndrome and mutations in *VHL* has been described in the pancreatic cysts obtained from patients with VHL syndrome[29]. The association of *VHL* mutations with SCN's has interesting implications. For one, SCN's are found in as many as 15% hereditary VHL syndrome along with other tumors like renal clear cell carcinoma. In fact, Pdx1-Cre; *Vhlh*^{lox/lox} mice with pancreas specific *VHL* deletion infrequently develop cystic adenomas in pancreas [30]. *VHL* mutations leads to upregulation of vascular endothelial growth factor (VEGF)-1, hypoxia-inducible factor 1 alpha (HIF1- α), carbonic anhydrase IX (CAIX) and glucose transporter (GLUT)-1. This may be responsible for the clear cell nature of and the prolific angiogenesis in the tumors in *VHL* syndrome. Intriguingly, the four samples from SCA patients which did not harbor a clear *VHL* mutation had lost one allele of chromosome 3p within or adjacent to *VHL* gene. It is possible that in these patients *VHL* gene was inactivated by genetic alterations such as deletions or translocations which will not be detectable by methods employed in the study. In this study the authors were also able to identify *VHL* mutations in the fluid from 50% of patients with SCAs. *TBC1D3* was the other gene mutated in 2 of the eight samples in this series. *TBC1D3* is a member of the TBC1 domain family and encodes for the GTPase-stimulating proteins. While application of *TBC1D3* is described in prostate cancer, the significance of this gene in the pathogenesis of SCAs is unknown.

It must be noted that the genetic alterations seen in pancreatic ductal adenocarcinoma (*KRAS*, *TP53*, *SMAD4*), IPMNs (*KRAS*, *GNAS*, *RNF43*) and MCNs (*KRAS*) are not seen in SCAs. This not only sheds light on unique pathogenesis of these cysts but also has clinical significance as this unique profile can be used to diagnose these cysts in patients where the radiologic findings are equivocal, and thus prevent un-necessary resection of asymptomatic SCAs. Springer et al [31] did a mutational analysis of cyst fluid and found that VHL mutations were detectable in 42% of SCAs; with 64% them demonstrating LOH at VHL locus and 8% of showing aneuploidy at 3p locus. Other SCA's didn't have any significant mutation. The authors' analytic algorithm found out that an absence of *KRAS*, *GNAS*, *RNF43* mutations, or lack of aneuploidy in 5p or 8p loci helped predict the presence of SCA with high sensitivity and specificity.

Lessons from Mouse Model

Mouse models help understand the critical role of the genetic mutations in the pathogenesis of neoplastic disease. Further, development of such models also provide a platform for basic and translational research into the pathogenesis and therapeutics of the disease. While a lot of progress has been made in the generation of genetic models of pancreatic carcinogenesis, there has been dearth of valid models of cystic neoplasms of the pancreas. However, with the new found understanding of the genetic makeup of pancreatic cystic neoplasms, new models are being increasingly described. As discussed above, there has been increasing understanding of the role of *GNAS* in the development of IPMNs. To investigate the role of *GNAS*, Taki et al [32] generated conditional transgenic mouse lines where, in the presence of Cre recombinase, wild type *GNAS* or *GNAS* with R201H mutation was expressed. These mice when crossed with *Ptfa^{cre/+}* expressed WT or mutated *GNAS* in pancreatic progenitor cells. Interestingly, in this model induction of mutated *GNAS* led to ectasia of main pancreatic duct, fibrosis and acinar atrophy which could represent an early phenotype of IPMN. However, these mice did not develop macroscopic tumors when followed for up to 2 months. This again supports that while *GNAS* mutation may be necessary, it may not be sufficient to develop full blown IPMN. The authors also evaluated the effect of introduction of *KRAS^{G12D}* in addition to *GNAS* in a pancreas specific manner by crossing the abovementioned mice with *LSL-Kras^{G12D}* mice. Interestingly, *Tg(CAG-LSL-GNAS^{R201H}); LSL-Kras^{G12D}; Ptf1aCre/+* mice developed ascites and pancreatic tumor starting 5 weeks of age and these mice had significantly shortened life span compared to the parent mouse lines. On histopathology, the tumors had typical IPMN-like features: dilated ducts, low to high grade papillae consisting of epithelial cell and extensive inflammation. Notably, like gastric or pancreatobiliary IPMNs, papillae showed strong positive staining for mucus core protein (MUC)1 and MUC5AC and phosphorylated substrates of Protein Kinase A. While ~50% of patients may have both *KRAS* and *GNAS* mutations, this model may not recapitulate biology of ~50% of patients who do not have both *KRAS* and *GNAS* mutation.

Activating *KRAS* mutations alone faithfully recapitulate PanIN lesions but do not lead to development of pancreatic cysts by themselves and additional mutations are needed. Siveke et al, overexpressed TGF α (driven by elastase) in addition to *KRAS^{G12D}* in the pancreas (mice expressing cre under control of *p48*) and observed that this led to accelerated PanINs formation as well as development of cystic neoplasms reminiscent of human IPMNs. These

cystic lesions contained mucin and were positive for MUC1 and 5AC, thus mimicking the pancreatobiliary subtype of human IPMNs. Interestingly, a significant number of these animals went on to develop invasive disease from both PanINs and IPMN lesions, as seen in human disease. Similar to that observed in human IPMNs[33], activation of several EGFR dependent oncogenic pathways was observed in this mice model. However, activating mutations of TGF α have not yet been shown to be present in IPMNs in humans so the relevance of this model to human disease is unclear.

To study the role of TGF- β in pancreatic carcinogenesis, Vincent et al knocked out transcription intermediary factor 1-gamma gene (*Tif1 γ*) in the pancreas of mice with constitutively active *KRAS*. This model is characterized by abundant PanIN's early on which are soon replaced by numerous cystic lesions. While there was a latency of ~ 7weeks in development of these cystic lesions, the penetrance was 100%. Many of the cells lining these cysts stained positively for cytokeratin (CK)19 as well as Alcian blue suggesting a mucus secreting ductal origin of these lesions. In the absence of an ovarian-type stroma, the lesions are more homologous to the human IPMN. Interestingly, IPMN-like lesions in this mouse model also have an endocrine component. Uncharacteristically, none of the IPMN's progressed into PDAC in the mice at the end of 13 weeks and while *Tif1 γ* expression levels are reduced in pancreatic cancer, mutations in this gene have not been observed in IPMNs. The significance of these mutations are yet to be fully explored in terms of human disease.

An important mouse model of MCN's was developed by Izeradjene et al in the form of a *KRAS* *LSL*^{G12D/+}; *Dpc4*^{flox/+} *p48*^{Cre/+} (KD) mouse. While the *KRAS* mutation induced low-grade PanIN's, the body and tail of the pancreata of these mice had grossly palpable cysts that could be 2–3 mm in size and are full of serous and/or hemorrhagic fluid. There were fewer metastases seen in this model compared to the KPC mouse model, an extensively described genetic model of PDAC. The cyst walls were characterized by columnar, CK19 and Alcian blue positive mucin- secreting epithelial cells without any papillae as seen in IPMN's. Additionally, the stroma of these cysts was characteristic ovarian-type and consisted of desmin and SMA expressing spindle shaped cells with wavy nuclei, both of which are hallmarks of MCN's. *KRAS*^{LSL-G12D/+}; *Dpc4*^{flox/flox} *p48*^{Cre/+} (KDD) mouse with both *Dpc4* alleles deleted have an even more aggressive tumorigenic phenotype (average survival of 8 months vs. 15 months in KD mouse). KDD mice develop extensive PDAC lesions through a probable MCN to PDAC route. These lesions had upregulated expression of Egfr, Her2/neu, Shh and Hes1. Compared to cell lines derived from KPC mice tumors, fewer cell lines could be successfully derived from KD mice and had a significantly higher doubling time. Also, these cell lines showed evidence of greater genomic stability than cell lines derived from KPC mice. The transcription of *p16*^{Ink4a} was frequently lost in many derived cell lines and was associated with a wild type *Trp53*. A major mechanism underlying such gene silencing was found to be epigenetic and in some cases, genomic deletion, where *p16*^{Ink4a} locus was frequently methylated and silenced. Phenotypically, KD or KDD cell lines showed differential effects to TGF β stimulation compared to KPC cells. In KD cells, TGF β didn't increase the motility of cells as much as in KPC. Importantly, this model helps in elucidating a pathway from MCN's to PDAC that is different from the classical one and depends on timing of the *DPC4* mutation. It appears that, mutation in *TRP53* and/or *p16*, after initiating *KRAS*^{G12D} mutation, leads the tumor cells down a

canonical PDAC fate, and an additional *DPC4* mutation only makes the cancer more aggressive. In contrast, a silencing mutation in *DPC4* early on, i.e. after *KRAS*^{G12D} leads the cell to a MCN phenotype with decreased proliferation and migration.

Clinical Implications and Future Directions

The goal of gaining a detailed understanding of the pathogenesis of neoplastic cysts and the pathways leading to cancer development is to 1) employ -omics approach to help in correctly diagnosing the type of cyst; 2) help in detecting if cancer has already developed in these lesions thus providing a clear indication for surgical approach; 3) predict of the biology of lesions with respect to progression to invasive lesions; and 4) attain the elusive goals of developing pharmacotherapy which may prevent development of invasive lesions. Typically, the correct diagnosis with respect to the nature of the cyst is made with help of cross-sectional imaging, endoscopic ultrasound with cyst fluid analysis including carcinoembryonic antigen (CEA) and cytology[4]. To improve the diagnostic and predictive ability, investigators have turned to molecular analysis. Initial studies involving the molecular markers in the cyst fluids have focused on predicting the risk of malignancy in mucinous cysts with high CEA. To this end the studies suggest that increased DNA content, presence of *KRAS* mutation, or two or more loci of LOH were associated with mucinous neoplasms and that *KRAS* mutation followed by allelic loss is a strongly predictive of a malignant cyst[34]. Based on these findings a molecular test PathFinder TG® is being increasingly promoted, though with only marginal, if any benefit in surgical decision making. In a recent study Springer et al[31] performed cyst fluid analysis to identify mutations in *BRAF*, *CDKN2A*, *CTNNB1*, *GNAS*, *KRAS*, *NRAS*, *PIK3CA*, *RNF43*, *SMAD4*, *TP53* and *VHL*, to identify LOH at *CDKN2A*, *RNF43*, *SMAD4*, *TP53* and *VHL*, and to identify aneuploidy and this information was used to select markers that could classify cyst type and grade. This study was based on the previous elucidation of genetic makeup of SCA, MCN, IPMN and solid pseudopapillary neoplasm[5, 7] thus highlighting the importance of defining the pathogenesis and progression pathways. The authors then used multivariate organization of combinatorial alterations to identify composite molecular markers to help correctly diagnose the cyst subtype as well as the cysts which need surgical resection. This study suggested that combination of molecular markers including presence of *VHL* mutation or chr3 LOH and/or absence of *KRAS*, *GNAS*, *RNF43* mutations, chr5p and chr8p aneuploidy detected SCA with 100% sensitivity and 91% specificity. Combination of absence of *CTNNB1*, *GNAS* mutations, chr3 LOH and chr1q and chr22q aneuploidy suggested a diagnosis of MCN with 100% sensitivity and 75% specificity. Presence of any of the following: *GNAS*, *RNF43*, chr9 LOH, chr1q and chr8p aneuploidy detects IPMN with 76% sensitivity and 97% specificity. Addition of clinical parameters increased sensitivity while decreasing specificity. More importantly the authors used a combination of molecular markers to predict need for surgical resection, i.e. identification of MCN, IPMN with high grade dysplasia or invasive component or solid pseudopapillary neoplasm. A combination of mutations in *SMAD4*, *TP53*, chr17 LOH (region containing *RNF43*) or aneuploidy in chr5p, 8p, 13q or 18q, chr17 LOH was used as a marker of IPMN with high grade dysplasia or invasive component. These mutations along with the molecular markers for MCN and solid pseudopapillary neoplasm were used to evaluate the ability of molecular diagnostics to

correctly identify the lesions needing surgical resection. The study demonstrated that the combination of these molecular markers correctly identified patients requiring surgical intervention with 75% sensitivity and 92% specificity. This study suggests that sometime in the near future, molecular diagnostics may help in clinical decision making in pancreatic cyst evaluation.. However, for now these molecular features only identify cysts which need surgical intervention at the time of evaluation. They do not predict the progression and evolution of these cysts. Future studies will be needed to identify markers of progression of dysplasia or even development of invasive component.

Conclusion

The current epidemic of pancreatic cystic neoplasms is a difficult clinical by-product of our expanding diagnostic capabilities. They range from asymptomatic benign incidental lesions to rapidly growing tumors with significant malignant potential. As such, diagnosing and managing pancreatic cystic neoplasms is a challenging clinical exercise in the absence of reliable markers of malignant progression and as yet an incomplete understanding of its pathophysiology. Recent efforts from clinical studies have identified key molecular driver mutations like KRAS and GNAS as early events. These findings along with mouse models that recapitulate morphological and natural history of progression from cystic lesions to malignancy is helping paint a clearer picture of this disease. Future studies will integrate our understanding of molecular changes in benign versus pre-malignant cysts to prognosticate and manage patients effectively.

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Figure 1A

Figure 1B

Figure 1.

Figure 1A. Mutational pathogenesis of IPMN. IPMNs are believed to arise from pancreatic ductal cells which acquire a mutation in either *KRAS* or *GNAS* in most cases. Intestinal IPMNs tend to have a higher frequency of *GNAS* mutations compared to pancreatobiliary IPMNs which give rise to colloid and tubular forms of invasive cancer respectively. The progression from a low grade lesion to an invasive phenotype occurs through accumulation of mutations in *RNF43*, *P16/INK4A*, *SMAD4* and *TP53*.

Figure 1B. Mutational pathogenesis of MCN and SCN. Most Mucinous cystic adenomas harbor early *KRAS* mutation and malignant lesions accumulate abnormalities in *TP53* and *SMAD4*. Serous cystadenomas result from an abnormalities (either LOH or allelic deletion) in *VHL* gene.