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Pancreatic DCLK1+ Cells Originate Distinctly from PDX1+ Progenitors and Contribute to the Initiation of Intraductal Papillary Mucinous Neoplasm in Mice

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Abstract

PanINs and IPMNs are the two most common precursor lesions that can progress to invasive pancreatic ductal adenocarcinoma (PDA). DCLK1 has been identified as a biomarker of progenitor cells in PDA progressed from PanINs. To explore the potential role of DCLK1-expressing cells in the genesis of IPMNs, we compared the incidence of DCLK1-positive cells in pancreatic tissue samples from genetically-engineered mouse models (GEMMs) for IPMNs, PanINs, and acinar to ductal metaplasia by immunohistochemistry and immunofluorescence. Mouse lineage tracing experiments in the IPMN GEMM showed that DCLK1$^+$ cells originated from a cell lineage distinct from PDX1$^+$ progenitors. The DCLK1$^+$ cells shared the features of tuft cells but were devoid of IPMN tumor biomarkers. The DCLK1$^+$ cells were detected in the earliest proliferative acinar clusters prior to the formation of metaplastic ductal cells, and were enriched in the “IPMN niches”. In summary, DCLK1 labels a unique pancreatic cellular lineage in the IPMN GEMM. The clustering of DCLK1$^+$ cells is an early event in Kras-induced pancreatic tumorigenesis and may contribute to IPMN initiation.

Keywords

Pancreatic IPMN; DCLK1; SOX9; KLF4

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CONFLICTS OF INTEREST STATEMENT

There is no conflict of interest pertaining to this publication to be disclosed by any of the authors.

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1. Introduction

Doublecortin CaM kinase-like-1 (DCLK1, previously referred to as DCAMKL-1) is originally identified in the developing nervous system [1, 2]. Its expression is one of the molecular features of gastrointestinal tuft cells [3, 4]. DCLK1 regulates the polymerization of microtubules in many mammalian cells by its N-terminal microtubule binding domain [5]. Its C-terminal kinase domain can be cleaved and released from the microtubule anchorage domain by cysteine protease calpain [6]. In addition, DCLK1 has recently been demonstrated as a putative biomarker of tumor-initiating cells in genetically engineered mouse model (GEMMs) for various types of tumors, including pancreatic cancer[7–12]. For example, DCLK1-expressing cells in intestinal tumors have been thought to be tumor progenitor cells, as diphtheria toxin-mediated ablation of DCLK1$^+$ cells led to tumor regression [7, 10]. However, in human gastrointestinal tumors, DCLK1-expressing cells were mainly observed at the early stages of tumorigenesis such as metaplastic and hyperplastic lesions [4, 13]. Increasing evidence also exhibit that DCLK1 might be a differentiated marker of mature tuft cells in the intestine, rather than a stem or progenitor cell marker in normal and tumor tissues [14, 15].

Pancreatic intraductal papillary mucinous neoplasms (IPMN) is one of the three known precursors of invasive pancreatic ductal adenocarcinoma (PDA). High frequency of DCLK1$^+$ cells has been also reported in precursor lesions of pancreatic cancer such as pancreatic intraepithelial neoplasias (PanINs) in both human and mouse [8, 15–17]. These morphologically tuft-like DCLK1-expressing cells have been recognized as putative stem/progenitor cells in pancreatic tissues of these GEMMs with PanIN/PDA histologic presentation [18], but how DCLK1$^+$ cells are involved in the genesis of pancreatic IPMNs has not been explored.

TGF-β superfamily signaling plays a pivotal role in determining the pathogenesis of PDA derived from PanINs or IPMNs [19–22]. We have reported that mutant *Kras* in the context of inactivated activin signaling promotes the development of IPMN/PDA in our recently established *Acvr1b*$^{flox/flox}$;*LSL-Kras*^{G12D};*Pdx1-Cre* GEMM [22, 23]. In contrast, mPanIN/PDA pathogenesis is the major histologic presentation in the *LSL-Kras*^{G12D}; *Pdx1-Cre* GEMM with or without additional *p16* inactivation [24, 25]. Using these established GEMMs with specific IPMN or PanIN genesis, we observed that DCLK1$^+$ cells were predominately detected in the pancreatic tissues with activated mutant *Kras* and not in the Cre-negative normal control mice. Pancreatic DCLK1$^+$ cells shared the molecular features of intestinal tuft cells but not the IPMN tumor cells. Lineage tracing demonstrated that these pancreatic DCLK1-expressing cells originated from cell lineage distinct from PDX1$^+$ progenitors. Furthermore, DCLK1$^+$ cells could be detected in the early stage of tumorigenesis, such as in the proliferative acinar clusters prior to the formation of metaplastic ductal cells, and were further enriched at the base of IPMN tumors.

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2. Materials and methods

2.1 Mouse strains

All animal experiments described here were approved by Columbia University Animal Care and Use Committees. LSL-KrasG12D;Pdx1-Cre (thereafter called KP) mice [24] with full spectrum of PanINs and low progression to invasive PDA were used as the representative PanIN model in this study. *Acvr1b*$^{flox/flox}$*; LSL-Kras*^{G12D}*; Pdx1-Cre* (thereafter called AKP) mice [22] (backcrossed to C57BL/6 background), a recently characterized GEMM for IPMN, were bred into *R26R*^{EYFP} mice (*B6.129X1-Gt(ROSA)26Sortm1(EYFP)Cos/J*, Jackson Laboratories) [26, 27] to trace the IPMN tumor cells by labeling the PDX1-expressing pancreatic epithelial cells with YFP protein. In the AKP GEMM, IPMNs are defined as mucinous cystic papillary proliferations involving main or branch ducts with a diameter larger than 1mm [22]. *MT-TGF-α* mice were treated with 25 mM of ZnSO4 in drinking water for eight months to induce the formation of acinar-to-ductal metaplasia (ADM) [28, 29]. Cre negative sibling mice of various genotypes were used as normal controls. To explore whether bone marrow-derived cells contributed to the genesis of pancreatic DCLK1-expressing cells, female KP mice aged 4 to 6 weeks old were irradiated and transplanted with bone marrow cells generated from male C57BL/6 or *Rag2*$^{-/-}$*;Il2rg*$^{-/-}$*;eGFP*$^{+/+}$ immunodeficient mice (a gift from Dr. Jessica Kandel, University of Chicago) [30].

2.2 Human Samples

The acquisition of the tissue specimens was approved by the Columbia University Institutional Review Board and performed in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations. All samples were selected from pancreatic resections performed at Columbia University Presbyterian Hospital between 2006 and 2008. By definition, all IPMN tissues utilized in the study involved the main pancreatic duct and/or branches. Histologic typing of the tumors was performed according to the recommendations in the WHO classification [31].

2.3 Immunostaining

Unstained 5-micron sections derived from the formalin-fixed and paraffin-embedded blocks were deparaffinized and hydrated by routine procedures. Sodium citrate buffer (pH 6.0) was used as the antigen retrieval. The primary antibodies at diluted concentrations were incubated overnight at room temperature. The primary antibodies are listed in Supplementary Table 1. For immunohistochemistry (IHC), the secondary antibodies used were Dako LSAB+system-HRP (universal) and Envision+system-HRP (anti-rabbit and anti-mouse polymer-HRP). For double IHC, anti-rabbit and anti-mouse polymer-AP kits were purchased from Vector (MP-5401 and MP5402), including the substrate kits for red peroxidase (SK-4805), red alkaline phosphatase (SK-5100) and blue alkaline phosphatase (SK-5300). For immunofluorescence (IF) assay, the fluorophore-conjugated secondary antibodies (Jackson ImmunoResearch) were incubated at room temperature for 2 hours. All other procedures were done according to the manufactures' instructions. The percentage of DCLK1+ cells for each experimental group (ADM, PanIN, IPMN, and normal) was determined by counting DCK1+ cells and total ductal epithelial cells present in the pancreata

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of ten randomly chosen mice within each group (MT-TGF-α, KP, AKP GEMM, and Cre-negative control), and at least three different sections of each individual pancreas were examined.

3. Results

3.1 DCLK1+ cells significantly accumulated in the precursor lesions of pancreatic tumors

We have previously reported a GEMM for IPMNs (*Acvr1b*$^{flox/flox}$;*LSL-Kras*^{G12D};*Pdx1-Cre* or the AKP GEMM) [22] which was generated by tissue-specific and conditional inactivation of the *Acvr1b* gene (*Acvr1b*$^{flox/flox}$) [23] in the pancreas in the context of oncogenic Kras activation (*LSL-Kras*^{G12D};*Pdx1-Cre* or the KP GEMM) [24]. To explore the potential role of DCLK1-expressing cells in the genesis of IPMNs, DCLK1-expressing cells were investigated in the pancreatic tissues of the AKP GEMM by immunohistochemistry (IHC) with the antibody against the C-terminus of DCLK1 protein. Pancreata from the well-established GEMMs for PanINs (the KP GEMM) and acinar-to-ductal metaplasia (*MT-TGF-α*) [28, 29], as well as the Cre–negative normal controls were examined for comparison. DCLK1$^+$ cells were frequently detected in the oncogene-induced precancerous lesions such as ADM, PanINs and IPMNs from the *MT-TGF-α*, KP, and AKP GEMM respectively (Fig. 1a). The percentage of DCLK1$^+$ cells in the ductal epithelial cells within each ductal lesion was not statistically different among the ADM (7.5±3.0%), PanIN (7.4±2.9%), and IPMN (6.5±1.7%) lesions (Fig. 1b). In contrast, no DCLK1$^+$ cells were detected in the normal controls examined. This absence of DCLK1$^+$ cells in the pancreatic ducts of the normal control is consistent with the previous observation [15, 17]. However, in the KP and AKP GEMMs, DCLK1$^+$ cells were detected in morphologically normal pancreatic ducts of these *Kras*^{G12D}-driven GEMMs [16] (Fig. 1c). The DCLK1$^+$ cells were particularly prevalent in KRAS-induced hyperplastic lesions, such as in the metaplastic ducts of ADM in the KP GEMM and IPMN lesions in the AKP GEMM (Fig. 1d and e). These findings suggest that the initiation of DCLK1$^+$ cells was probably one of the earliest events in oncogene-induced pancreatic tumorigenesis.

In our mouse AKP mIPMN model, the interspersed DCLK1$^+$ cells morphologically resembled intestinal tuft cells, which typically display a narrow apical region [32] (Fig. 1e). Unlike mIPMN tumor cells, DCLK1-expressing cells lacked the ductal differentiation marker CK19 (Fig. 1f and S1a). The paucity of mucinous granules in the DCLK1$^+$ cells was also easily discernible from the mucin-abundant IPMN tumor cells by Alcian blue and PAS staining (Fig. S1b and c). The double IHC confirmed that DCLK1$^+$ cells did not express MUC5AC protein, a common biomarker for IPMN tumor cells (Fig. 1g). In addition, these DCLK1$^+$ cells co-expressed typical biomarkers of tuft cells, such as acetylated tubulin, phalloidin, and COX2 (Fig. 1h, k and i; S1d and e), indicating that DCLK1$^+$ cells in the mIPMN are tuft-characterized cells without typical epithelial tumor biomarkers.

3.2 DCLK1 marked a distinct cell lineage from tumors cells in murine IPMN

In our AKP GEMM, the mIPMN lesions are mostly devoid of ACVR1B expression due to the engineered Pdx1-Cre-induced *Acvr1b* deletion in the pancreas [22]. However, we detected residual ACVR1B$^+$ cells by IHC in the mIPMN lesions. Those ACVR1B$^+$cells with

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DCLK1 expression are not only in a scattered-distribution pattern as in the intestine but also morphologically resemble tuft cells in the intestine (Fig. 2a). We randomly counted more than one hundred ACVR1B$^+$ cells in distinct individual pancreatic mIPMN lesions, and 100% were found co-expressed with DCLK1 by IHC analyses of serial sections. This result was further confirmed by double immunofluorescence (IF) demonstrating co-localization of ACVR1B and DCLK1 expression in the mIPMNs (Fig. 2b), indicating that the double ACVR1B/ DCLK1$^+$ cells identified within mIPMNs might not share the same cellular origin with PDX1$^+$-derived pancreatic mIPMN tumor cells (which were ACVR1B-null). To demonstrate that DCLK1$^+$ cells were not derived from PDX1$^+$ pancreatic progenitor lineages, we bred the *R26R^{EYFP}* mouse line into our AKP GEMM to enable YFP labeling of all pancreatic epithelial cells derived from PDX1$^+$ progenitors. The double IF demonstrated that all the YFP-labeled-IPMN tumor cells did not overlap with DCLK1 expression (Fig. 2c). To further investigate if DCLK1$^+$ cells might originate from the bone marrow, we examined five KP mice that had undergone bone marrow transplantation with GFP$^+$ bone marrow cells for DCLK1 expression in the pancreatic tissues. None of the DCLK1$^+$ cells in the pancreas were labeled by GFP (Fig. 2d), indicating that pancreatic DCLK1$^+$ cells were not bone marrow-derived.

In the AKP GEMM, the DCLK1-expressing cells within mIPMNs were negative for PDX1 expression by double IF (Fig. S2a), providing further evidence that they were not of the pancreatic epithelial cell lineage. The expression of oncogenic biomarkers such as EGFR, PIK3CA and HER2 (Fig. S2b–d) were also not detectable in DCLK1$^+$ cells located within the mIPMN lesions, supporting the observation that those pancreatic tuft-like DCLK1$^+$ cells were distinct from the mucinous tumor cells of IPMNs. Taken together, these data unequivocally demonstrated that DCLK1 cells expressed robust ACVR1B and did not originate from PDX1$^+$ compartments in the pancreas.

3.3 Clustering of pancreatic DCLK1$^+$ cells is early event during ADM process

In *Kras*-inducing pancreatic tumorigenesis, ADM is widely recognized as an initiating process of PanIN/PDA [33]. Proliferative acinar clusters, consisted of only amylase$^+$ acinar cells without CK19$^+$ ductal cells, arise prior to ADM. DCLK1-expressing cells were previously observed to be enriched in early stages of tumorigenesis such as ADM/PanINs [8, 15, 16], In the AKP GEMM, we found that DCLK1$^+$ cells were present earliest in the acinar clusters prior to the formation of a lumen (Fig. 3a and b). During the process of ADM, the DCLK1$^+$ cells were already detectable in the initiating acinar clusters prior to trans-differentiation to CK19$^+$ ductal cells (Fig. 3c and d). Based on this early involvement of DCLK1$^+$ cells in ADM formation, together with our previous demonstration that DCLK1$^+$ cells originate differently from PDX1$^+$ cell lineage, we hypothesize that trans-differentiating acinar cells induced by oncogenic KRAS might stimulate the development or recruitment of the DCLK1$^+$ cells in pancreas. The DCLK1$^+$ cells then in turn promote the process of acinar to ductal metaplasia (Fig. 3e).

Inflammation-induced metaplastic ductal cells may be reprogrammed back to acinar cells upon suspension of the acute inflammation stimulus. However, oncogenic *Kras*-driven acinar cell dedifferentiation typically proceeds in a one-way direction to metaplastic ductal cell and

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further neoplastic cell [34]. To investigate the inter-relationship between oncogenic *Kras*-induced metaplasia and *Kras*-promoted accumulation of DCLK1$^+$ in IPMN, we examined the embryonic signaling SHH in ADM lesions in the IPMN GEMM mice, because SHH has been reported to induce metaplasia of acinar to ductal cells [35]. Significant upregulation of SHH expression was noted in the metaplastic ductal cells (Fig. 3f) but not in the adjacent DCLK1$^+$ cells (Fig. 3g). DCLK1$^+$ cells within the ADM clusters were devoid of SHH expression. Therefore, *Kras*-promoted DCLK1$^+$ cells likely have played a supportive role by stimulating the adjacent acinar cells to express SHH. The upregulation of those embryonic molecular signaling pathways in acinar cells by DCLK1$^+$ cells may be one of the mechanisms for the initiation of ADM and promoting the development of IPMN tumors in our GEMM.

3.4 Pancreatic DCLK1$^+$ cells are critical cellular components in the initiating niches of IPMNs

Thus far our data supported a working model in which that pancreatic DCLK1$^+$ cells behave similarly to tuft cells in the intestine, not as direct tumor stem or progenitor cells in IPMN genesis. The stem cells of normal intestinal villus are located at the crypt bases that serve as its progenitor differentiation niche [36]. Intestinal tumorigenesis could be derived from the neoplastic stem cells or progenitors at those crypt bases [7, 37]. We also observed similar differentiation niches in pancreatic IPMNs. First of all, we noted that DCLK1$^+$ cells in the AKP GEMM were predominantly concentrated in the mIPMN initiating niches such as the intestinal crypt-like lesions in the mIPMN tumors (Fig. 4a and b), the base of the mIPMNs (Fig. S3a and b), and the normal ductal junction of the tumors (Fig. S3c). Some isolated DCLK1$^+$ cells are also detected in low-grade mIPMNs (Fig. S3d). The epithelial ductal cells adjacent to tumors have been usually recognized to be hyperplastic and contain more immature and undifferentiated cells [38]. The clustering of DCLK1$^+$ cells at these “crypt-like” bases was also observed in human IPMNs (Fig. 4c). Secondly, we observed an increase in Ki67$^+$ cells in the vicinity of the DCLK1+ cells clustering in the mIPMNs, indicating the existence of the proliferative progenitor cells in these niches (Fig. S4a), in a similar distribution pattern of Ki67$^+$ cells observed in the crypt bases of intestinal villus (Fig. S4b). Importantly, none of the DCLK1$^+$ cells was found coexpressing Ki67 by using double IHC and counting more than one hundred DCLK1$^+$ cells in mIPMN lesions (Fig. 4d, S3e, S4c). This result supported that pancreatic DCLK1$^+$ cells in our IPMN GEMM are most likely quiescent and post-mitotic, distinct from those Ki67+ proliferative progenitor cells. Thirdly, several embryonic developmental signal pathways were activated or upregulated in the IPMN tumor cells along with clusters of DCLK1$^+$ cells, but not in DCLK1$^+$ cells themselves. For example, expression of β-catenin (Fig. 4e) and SHH (Fig. 4f) were found only in the mucinous tumor cells in the mIPMNs adjacent to enriched DCLK1$^+$ cells but not in DCLK1$^+$ cells themselves. Thus, pancreatic DCLK1$^+$ cells may serve as supportive cellular components in the “IPMN initiating niches” but are not tumor progenitor cells.

In addition, most of the clustered DCLK1$^+$ cells in mIPMNs exhibited strong nuclear SOX9 expression (Fig. 4g), although occasionally SOX9$^-$/DCLK1$^+$ cells were observed in the same mIPMN lesions (Fig. S3f). Cytoplasmic KLF4 expression was also detected in DCLK1$^+$ cells by IHC analysis of consecutive sections (Fig. S3g and h). The co-expression

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of KLF4 in DCLK1^{+} cells was confirmed by IF (Fig. 4h). Both SOX9 and KLF4 have been reported to be cell lineage transcription factor and potential biomarkers of gastrointestinal progenitors [39, 40], implying that pancreatic DCLK1^{+} cells could share the same cell origin with gastrointestinal progenitors, but not with pancreatic epithelial progenitors.

4. Discussion

DCLK1^{+} cells have been previously demonstrated being involved in both intestinal regeneration homeostasis and response to pancreatic inflammatory injury [7, 17]. The inflammation-induced regeneration seems to be essential for oncogene-related carcinogenesis [41]. The mechanisms for the interaction between oncogene-induced inflammation and tumorigenesis in pancreas are not clear. In this study, we showed that DCLK1^{+} cells are mainly enriched in inflammatory lesions ADM and precancerous lesions mIPMNs and mPanINs, and rarely detected in the pancreatic tissues of wild-type mice; exhibiting a preferential induction of DCLK1^{+} cell lineage by oncogenic Kras in pancreatic tissue (Fig. 1). Thus, the genesis of pancreatic DCLK1^{+} cells may provide a platform for studying the relationship between oncogene-induced inflammation and tumorigenesis. This study did not aim to define the cell of origin of IPMNs, but rather focus on the contribution of DCLK1^{+} to IPMN tumorigenesis.

Kras-induced oncogenesis targets either the terminally differentiated pancreatic cells or progenitors. In either case, the subsequently transformed tumor cells must contain genetically recombined floxed allele(s). Pancreatic DCLK1^{+} cells identified in the *Kras*-induced mIPMN tumors exhibited no evidence of genetic recombination, suggesting that these DCLK1^{+} cells might represent a unique pancreatic cell lineage in mice (Fig. 2). These *Kras*-induced DCLK1^{+} cells did not share the ductal biomarker CK19 or mucinous proteins of mIPMN tumor cells (Fig. 1f, g). But they exhibited the molecular characters of intestinal tuft cells (Fig. 1h, k and i; S1d and e). These data led us to investigate the potential role of these tuft-like DCLK1^{+}cells in pancreatic tumor initiation. Trans-differentiation of acinar to ductal cell induced by mutant Kras has been proposed to be the primary event in the PDA initiation [33, 41]. We found that pancreatic DCLK1^{+} cells were present even earlier than metaplastic ductal cells in *Kras*-induced ADM, supporting a role of DCLK1^{+} cells in the initiation of ADM process (Fig. 3). Again, DCLK1+-expressing cells were devoid of CK19 and amylase expressions (Fig 3c, d). In addition, overexpression of embryonic developmental markers such as SHH were noted in tumor cells but not in DCLK1^{+} cells (Fig. 3f, g, Fig. 4f). Previous study showed that pancreatic DCLK1^{+} cells could potentially be reprogrammed from acinar cells through metaplastic events under the oncogenic *Kras* stimulation [15]. However, our study demonstrated that these DCLK1^{+} cells were not derived from the PDX-1^{+} pancreatic progenitors, and might be first recruited to the pancreatic tissues with Kras activation, then played a supporting role in the Kras-induced acinar cell metaplasia.

In mIPMNs, pancreatic DCLK1^{+} cells were focally concentrated in metaplastic, hyperplastic and early dysplastic lesions, and but were rarely seen in invasive carcinoma, which is consistent with the previous observations in mPanINs [4, 8, 15]. Pancreatic DCLK1^{+} cells were particularly confined to the “IPMN niches”, which were characterized by the

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abundance of highly proliferative cells (Fig. 4, S3, S4). Our data and previous publications suggest that IPMN adenoma may grow papillae in similar manner as intestinal crypt does [42–45], with these highly proliferative Ki67$^+$ neoplastic progenitor cells clustering at the intestinal "crypt-like" bases in our AKP GEMM (Fig. S4). Significantly, none of the DCLK1$^+$ cells was found coexpressing Ki67 (Fig. 4d, S3e, S4c), which is consistent with the hypothesis that pancreatic DCLK1$^+$ cells serve as critical supportive cells constituting a cellular component of "IPMN niches" rather than serving directly as the tumor progenitor cells. In addition, pancreatic DCLK1$^+$ displayed nuclear SOX9 and cytoplasmic KLF4 expressions (Fig. 4g, h), which are biomarkers of gastric-intestinal progenitor lineage. Interestingly KLF4 has been demonstrated to play a critical role in early pancreatic tumorigenesis and ADM reprogramming [46]. Intestinal tuft cells originate from Lgr5-expressiong crypt base columnar stem cells like enterocytes, enteroendocrine, Paneth, and goblet cells, which are all cell components of the intestinal epithelium [7, 47, 48]. SOX9 is required for Paneth cell differentiation [49, 50], while KLF4 is required for terminal differentiation of goblet cells [51] in the intestinal epithelium. To unmask the functions of pancreatic DCLK1$^+$ cell in IPMN tumorigenesis, it would be of great interest to investigate the impacts of SOX9 or KLF5 inactivation to DCLK1$^+$ cells and the development of IPMN in our AKP GEMM in the future.

Taken together, DCLK1 labels a unique pancreatic cellular lineage in mouse. Pancreatic DCLK1$^+$ cells are rare in wild-type mice. The clustering of DCLK1$^+$ cells is an early event for oncogene-induced pancreatic tumorigenesis. Although pancreatic DCLK1$^+$ cells do not serve as tumor progenitor cells for IPMNs, they contribute to the initiation of IPMNs and remain potential targets for future therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- In this manuscript, we investigated the role of DCLK1$^+$ cells in the tumorigenesis of IPMN (intraductal papillary mucinous neoplasms). Pancreatic intraepithelial neoplasias (PanINs) and IPMNs are the two most common precursor lesions that can progress to invasive pancreatic ductal adenocarcinoma (PDA). Recently DCLK1 has been shown as a biomarker of progenitor cells in PDA progressed from PanINs.
- In contrast to PanIN, the development of IPMN is less understood and vastly understudied. We have previously published a novel genetically-engineered mouse model (GEMM) with the propensity of developing IPMN. Using this model and other established GEMMs that represent acinar-to-ductal metaplasia (ADM) and PanIN, we discovered that DCLK1$^+$ cells originated from a cell lineage distinct from pancreatic PDX1$^+$ progenitors.
- The pancreatic DCLK1$^+$ cells shared the features of tuft cells but were devoid of IPMN tumor biomarkers. This is in contrast to previous publications that DCLK1$^+$ serve as tumor stem cells for PanIN/PDA development.
- Although DCLK1-positivity doesn't serve as a stem cell marker for IPMNs, DCLK1$^+$ cells are important cellular components of "IPMN niches", and remain potential targets for future therapies.

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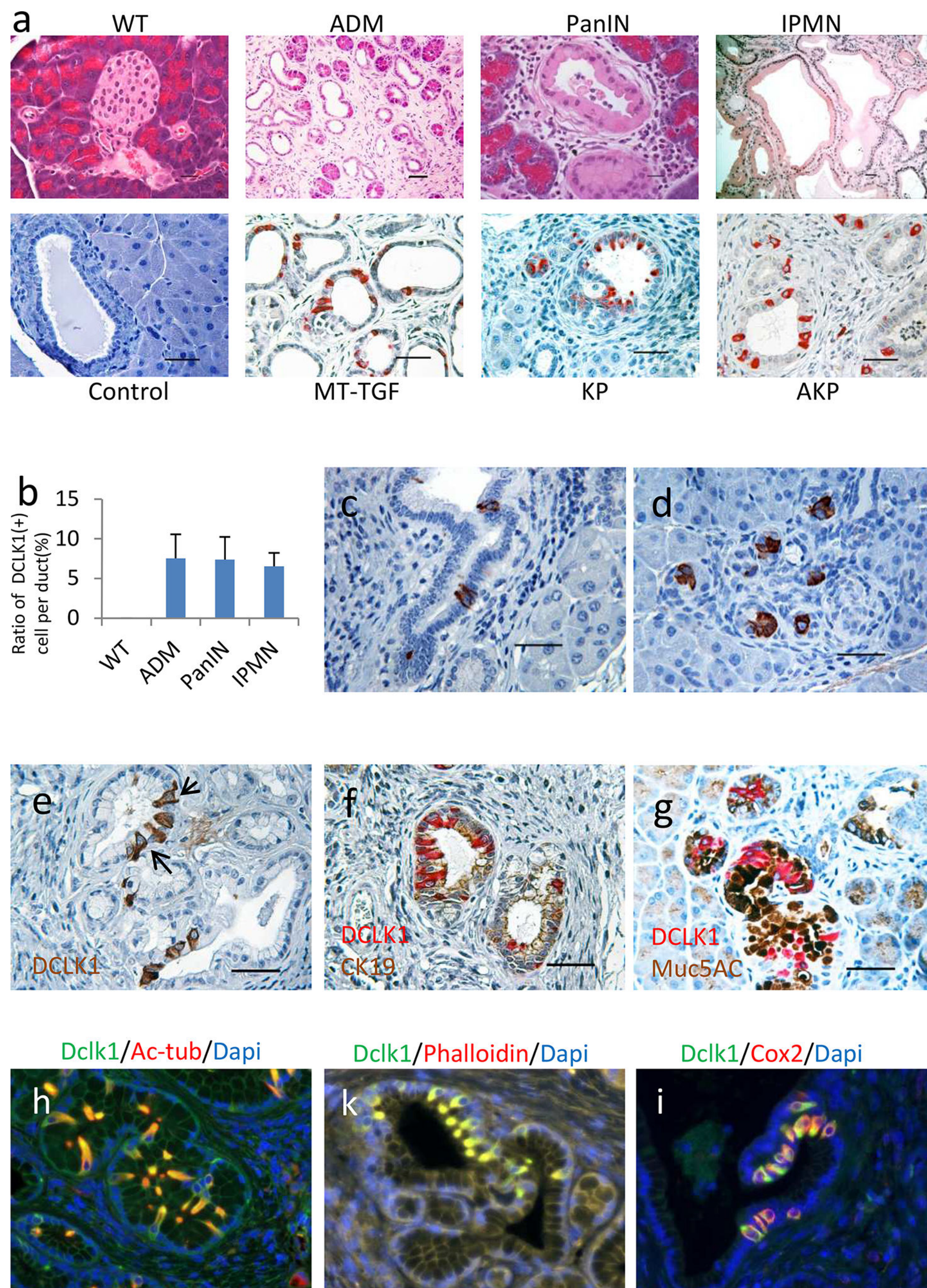

Fig 1. DCLK1$^+$ cells were enriched in oncogene-induced inflammatory ductal lesions

(a) DCLK1 positive cells(red) were focally detected in the ductal lesions of mouse pancreases with ADM (*MT-TGF-α* mice), PanIN (KP GEMM) and IPMN (AKP GEMM), but not in wild-type pancreatic ducts; (b) Quantitative analysis of average percentage of DCLK1$^+$ cells in total ductal epithelial cells per each duct in the pancreases with ADM (7.5±3.0%), PanIN (7.4±2.9%), IPMN (6.5±1.7%) and wild-type control (0) (n=3 in each group); (c) DCLK1-expressing cells (brown) were identified in the morphologically normal ducts of pancreases with mutant Kras activation;(d) Representative of mutant Kras-induced inflammatory nodules containing high frequency of DCLK1$^+$ cells; (e) DCLK1$^+$ cells

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(brown) in mIPMNs presented the typical morphological features of intestinal tuft cells with narrow apical side and wide basal bottom (arrow); Double IHC showed that $DCLK1^{+}$ cells (red) did not express the pancreatic epithelial cell biomarker CK19 (brown)(f) or IPMN mucinous protein MUC5AC (brown) (g); IF demonstrated that $DCLK1^{+}$ cells shared the same expression profile of tuft cells such as acetylated tubulin(h), phalloidin (k) and COX2 (i). Scale bars indicate 50μm.

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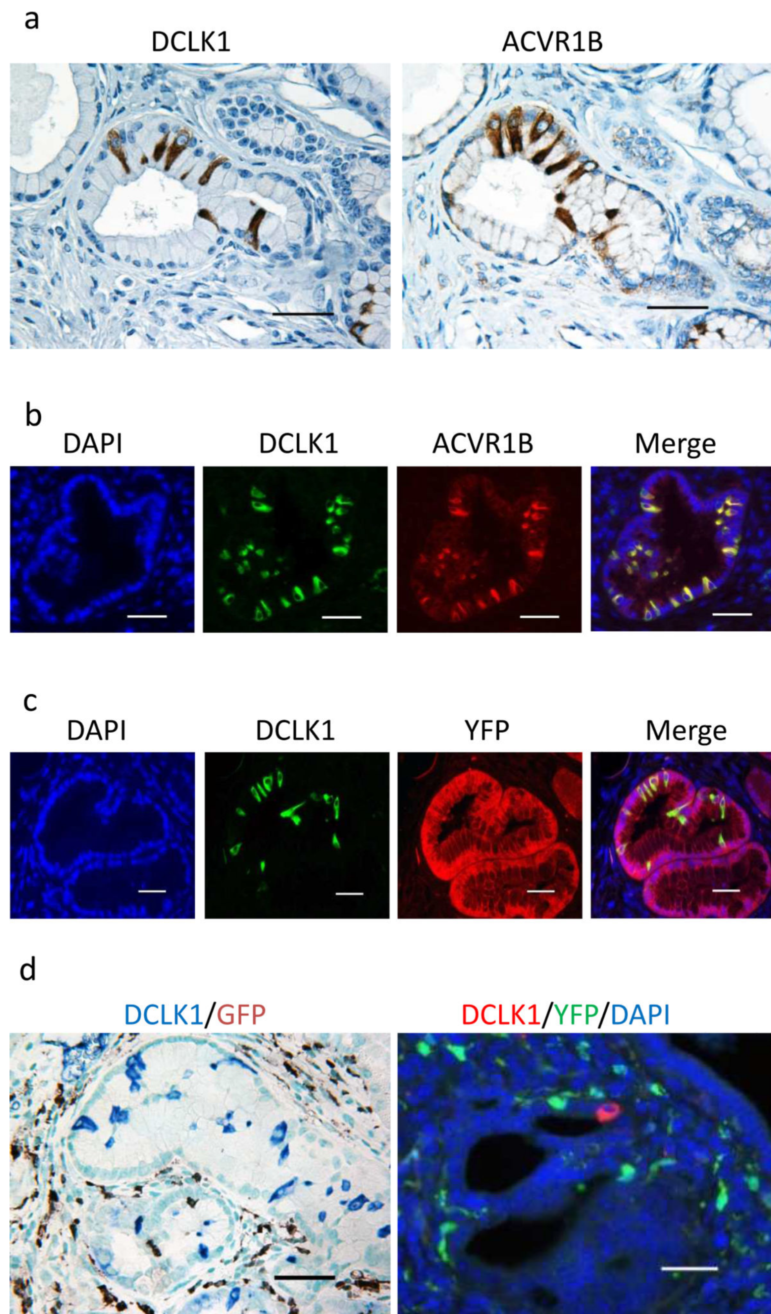

Fig 2. DCLK1 marks a unique pancreatic cell lineage in mouse

(a) ACVR1B protein presented in $DCLK1^{+}$ cells within the *ACVR1B* deleted mIPMN tumors; (b) Double IF confirmed co-expression of ACVR1B in all of $DCLK1^{+}$ cells in the *ACVR1B*-deficient IPMNs; (c) $DCLK1^{+}$ cell was not labeled with YFP in *Acvr1b*$^{flox/flox}$; *Kras*^{G12D}; *Pdx1-Cre* and *R26R*^{EYFP} mice (n=5); (d) $DCLK1^{+}$ cells were not derived from GFP^{+} bone marrow cells by bone marrow transplantation in pancreatic Kras mutant mouse model with double IHC (DCLK1 (blue), GFP (red), with methyl green counterstaining) and double IF (DCLK1/red; GFP/green) (n=2). Scale bars indicate 50μm.

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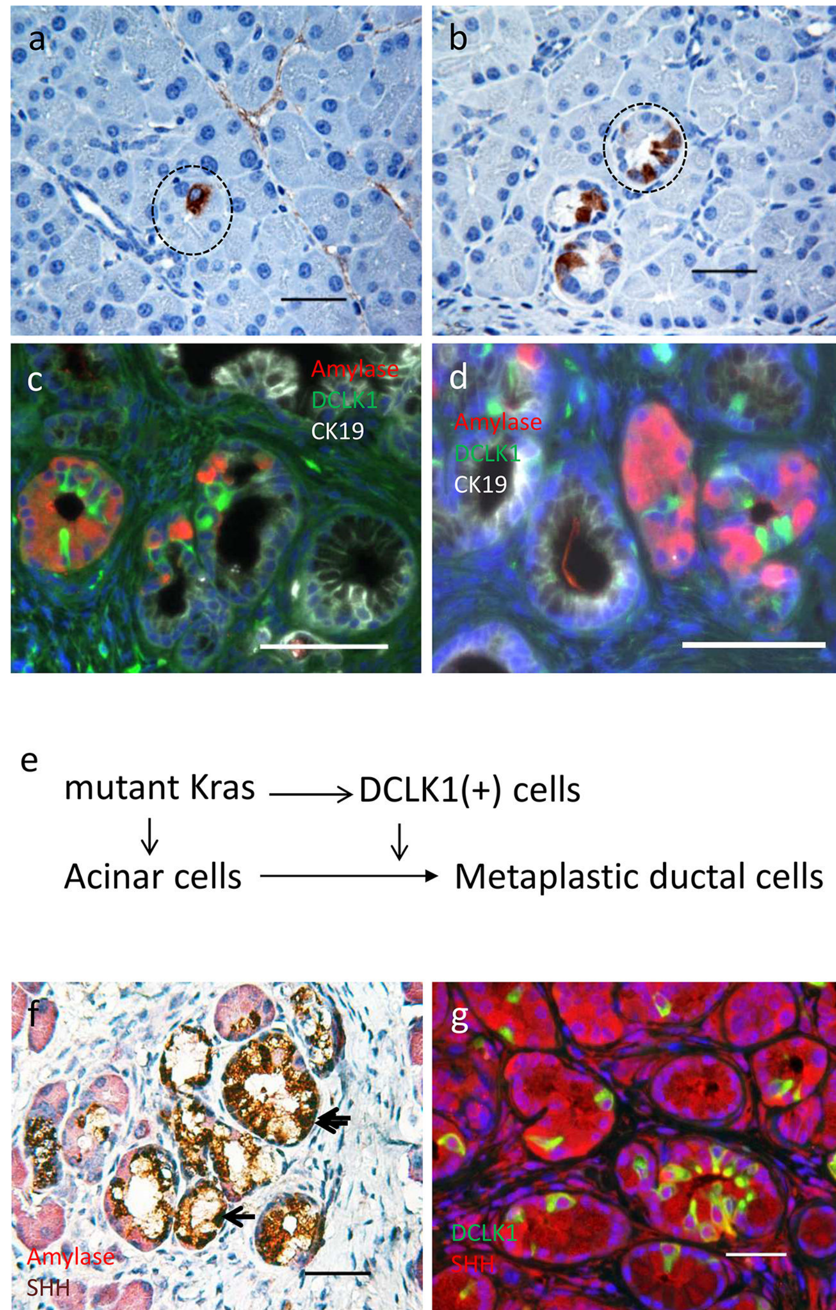

Fig 3. Pancreatic DCLK1$^+$ cells contribute to the ADM initiation

(a) DCLK1$^+$ cell appeared in the acinar clusters prior to formation of metaplastic ductal cells; (b) more DCLK1$^+$ than duct-like cells were observed in the initiating stage of ADM lesions; (c) and (d) triple IF confirmed that DCLK1$^+$ cells (green) prior to CK19$^+$ ductal cells (gray) presented in the initiating stages of ADM (arrow). Acinar cells were labeled with red; (e) two potential hypothesis relevant to the roles of pancreatic DCLK1$^+$ cells in ADM process; (f) SHH (brown) was dramatically upregulated in the metaplastic ductal cells (amylase/red) by double IHC (arrow); (g) double IF confirmed the significant

overexpression of SHH (red) in the metaplastic ductal cells but not the adjacent DCLK1^{+} cells (green) in ADM (arrow). Scale bars indicate 50 μm.

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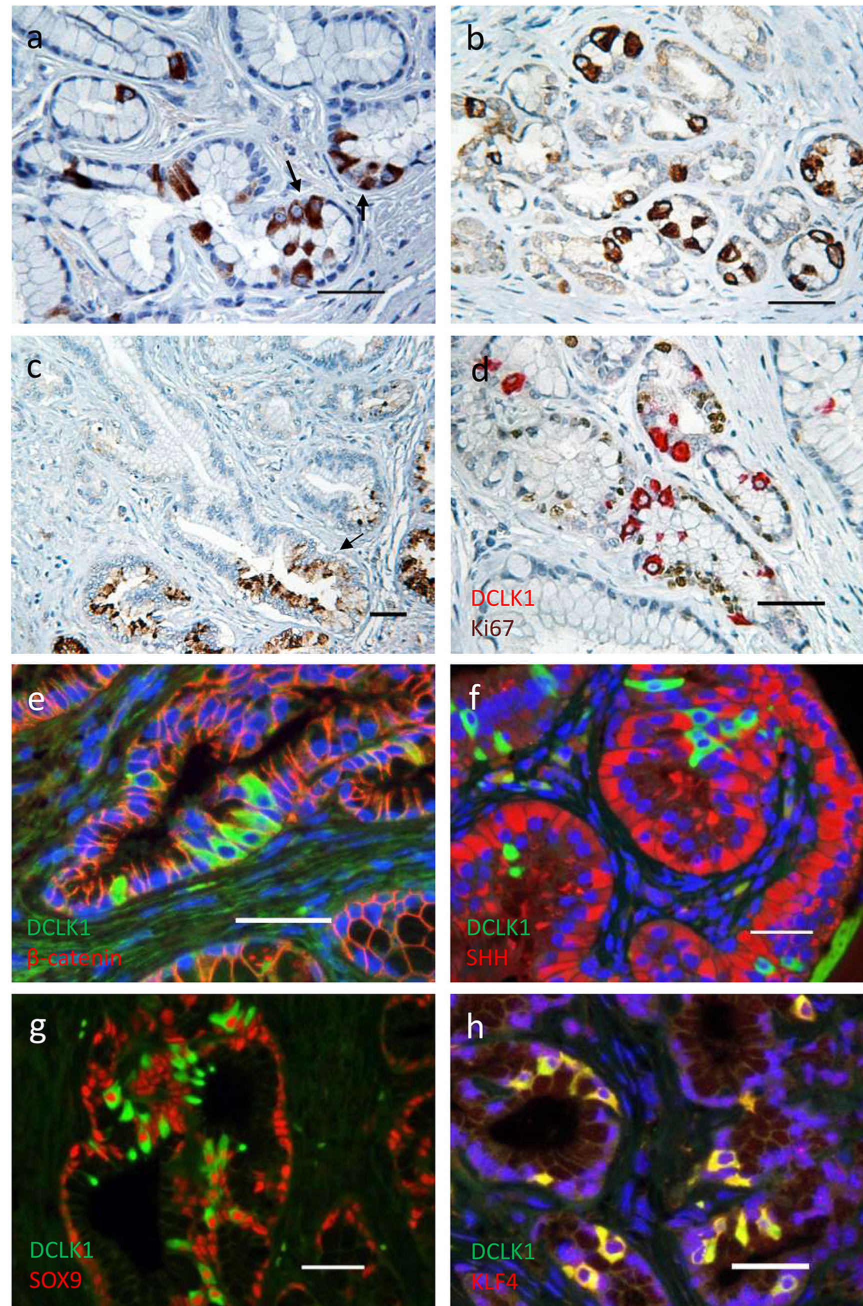

Fig 4. DCLK1$^+$ cell-enriched niches are present at the base of pancreatic IPMN tumors
DCLK1-expressing cells were enriched in the "crypts-like" of mIPMN proliferation (a and b) and human IPMNs (c); DCLK1$^+$ cell(red)-enriched-IPMN niches exhibited abundant compartment of Ki67$^+$ cells (brown) by double IHC(d); β-catenin (red) (e) and SHH (red) (f) were overexpressed in the DCLK1$^+$ cell(green)-enriched IPMN tumor cells; DCLK1$^+$ cells(green) presented commonly with intense nuclear SOX9 expression (red)(g); IF showed co-expression of KLF4 (red) in DCLK1$^+$ cells (green)(h). Scale bars indicate 50 μm.