Title
Atypical flat lesions derive from pancreatic acinar cells

Journal
Pancreatology, 17(3)

ISSN
1424-3903

Authors
von Figura, Guido
Fahrenkrog-Petersen, Leonie
Hidalgo-Sastre, Ana
et al.

Publication Date
2017-05-01

DOI
10.1016/j.pan.2017.04.014

Peer reviewed

HHS Public Access

Author manuscript

Pancreatology. Author manuscript; available in PMC 2018 May 01.

Published in final edited form as:

Pancreatology. 2017 ; 17(3): 350–353. doi:10.1016/j.pan.2017.04.014.

Atypical flat lesions derive from pancreatic acinar cells

Guido von Figura, MD[a,*], **Leonie Fahrenkrog-Petersen, MD**[a], **Ana Hidalgo-Sastre, PhD**[a], **Daniel Hartmann, MD, PhD**[c], **Norbert Hüser, MD**[c], **Roland M. Schmid, MD**[a], **Matthias Hebrok, PhD**[b], **Nilotpal Roy, PhD**[b,1], and **Irene Esposito, MD**[d,1]

[a]II Medizinische Klinik und Poliklinik, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany

[b]Diabetes Center, Department of Medicine, University of California, San Francisco, San Francisco, CA 94143, USA

[c]Chirurgische Klinik und Poliklinik, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany

[d]Institute of Pathology, University Clinic Duesseldorf, Heinrich-Heine University, Duesseldorf, Germany

Abstract

Objectives—Pancreatic ductal adenocarcinoma (PDAC) is thought to derive from different precursor lesions including the recently identified atypical flat lesions (AFL). While all precursor lesions and PDAC share ductal characteristics, there is an ongoing debate about the cellular origin of the different PDAC precursor lesions. In particular, pancreatic acinar cells have previously been shown to display a remarkable plasticity being able to undergo ductal dedifferentiation in the context of oncogenic stimuli.

Methods—Histological analyses were performed in a murine PDAC model that specifically expresses oncogenic Kras in adult pancreatic acinar cells. Occurrence, characterization, and lineage tracing of AFLs were investigated.

Results—Upon expression of oncogenic Kras in adult pancreatic acinar cells, AFLs with typical morphology and expression profile arise. Lineage tracing confirmed that the AFLs were of acinar origin.

Conclusions—Using a murine PDAC model, this study identifies pancreatic acinar cells as a cellular source for AFLs.

Keywords

Pancreatic cancer; AFL; IPMN; PanIN

[*]Corresponding author. II Medizinische Klinik und Poliklinik, Klinikum rechts der Isar, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Germany. gvfigura@tum.de (G. von Figura).

[1]These authors contributed equally.

Conflict of interest

The authors declare no conflict of interest.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest human cancers and is characterized by a poor survival rate. One of the main reasons for the dismal survival rate is that the diagnosis is often made at an advanced stage, making surgical intervention impossible. Therefore, there is an urgent need to better understand how PDAC initiates in order to develop novel diagnostic and screening tools. PDAC is known to arise from different precursor lesions, namely pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN) [1,2]. Recently, additional possible alternative PDAC precursors described as atypical flat lesions (AFLs) have been identified in a murine model of PDAC and in patients with familial pancreatic cancer [3]. AFLs are defined as ductal lesions that (i) arise in areas of acinar-to-ductal metaplasia (ADM), (ii) are highly proliferative, (iii) display a tubular structure, (iv) show cytological atypia, and (v) are typically surrounded by a loose but highly cellular stroma.

Although all these precursor lesions share a ductal morphology, studies in genetically engineered mouse models of PDAC have shown that selectively targeting the principal driver mutation of PDAC, an oncogenic version of Kras ($Kras^{G12D}$), to adult pancreatic acinar but not ductal or centroacinar cells results in the conversion of acinar to duct-like cells and the formation of PanIN lesions through a process termed acinar-to-ductal reprogramming (ADR) [4]. In contrast, further genetic changes, in addition to expression of oncogenic Kras, were necessary to elicit IPMN and PDA formation from duct cells in a transgenic mouse model [5]. Therefore, ductal PDAC precursor lesions can arise from different cell types, including duct and acinar cells.

AFLs have previously been shown to arise in mouse models of PDAC that target $Kras^{G12D}$ to embryonic pancreatic progenitor cells using $Ptf1a^{Cre}$ [3]. *Ptf1a* directs Cre activity during pancreatic organogenesis to all exocrine compartments, namely pancreatic ductal, centroacinar, and acinar cells [6]. Given this broad expression profile, a conclusion regarding the cellular origin of AFLs is not possible in this model. Using a mouse model that recombines $Kras^{G12D}$ specifically in adult pancreatic acinar cells but not in ductal or centroacinar cells, we show that AFLs with typical morphological, cytological and expression features arise from acinar cells. Thus, this study further highlights the importance of pancreatic acinar cells as a cellular source for PDAC.

Materials and methods

Animal experiments

The following mice were used to generate the experimental animals for this study and were maintained on a mixed genetic background: $Ptf1a^{CreER}$ [7], $LSL\text{-}Kras^{G12D}$ [8], and $R26R^{YFP}$ [9]. All studies on mice presented here were approved by the UCSF Institutional Animal Care and Use Committee (IACUC).

Tamoxifen was administered by oral gavage to 5 to 6-week-old mice as previously described [10] and mice were sacrificed at the indicated time points after tamoxifen induction.

Microscopy and immunohistochemistry

For quantification of ADM and AFLs, random whole pancreatic sections stained with hematoxylin and eosin H&E were analyzed. For immunohistochemical staining, sections of paraffin embedded pancreata were rehydrated and antigen retrieval was performed using Antigen Unmasking Solution (Vector Laboratories). Overnight incubation with the following primary antibodies was done at 4°C: goat anti-CPA1 (R&D), rabbit anti-CK19 (Abcam), rabbit anti-GFP (Santa Cruz Biotechnology), mouse anti-Ki67 (BD), rabbit anti-P53 (Vector Laboratories), goat anti-Amylase (Santa Cruz Biotechnology), rabbit anti-p44/p42 MAPK (Cell Signaling) and FITC-conjugated DBA-lectin (Vector Laboratories). Biotin-conjugated secondary antibodies were incubated for 1 h at room temperature, following development with ABC and DAB kits (both Vector Laboratories). Nuclear counterstaining was performed using haematoxylin. For immunofluorescence, sections were incubated with fluorophore-conjugated secondary antibody for 1 h at room temperature. Slides were mounted with DAPI hardset antifade mounting medium.

Statistics

The statistics for the correlation of ADM and AFLs were performed using linear regression in Prism V6.0 (GraphPad Software Inc.). Quantification of YFP-positive cells was done using ImageJ. Five random fields from each mouse pancreas (n = 3 mice) were chosen for quantification. T-test was performed using Microsoft Excel.

Results

Atypical flat lesions arise from pancreatic acinar cells

To determine whether AFLs can arise from pancreatic acinar cells, $Kras^{G12D}$ was specifically expressed in adult pancreatic acinar cells using the $Ptf1a^{CreER}$ allele. In contrast to $Ptf1a^{Cre}$, this model allows Cre-mediated recombination upon tamoxifen administration exclusively in adult pancreatic acinar cells as previously reported [4,7]. First, we confirmed that $Ptf1a^{CreER}$ specifically recombines in acinar but not centroacinar or ductal cells, in our hands, using the lineage tracing allele $R26R^{YFP}$ (Supplementary Fig. 1). Next, we analyzed pancreatic abnormalities in $Ptf1a^{CreER}$; $LSL\text{-}Kras^{G12D}$ mice. Of note, we identified areas of ADM and PanINs and also observed AFLs within ADM areas (Fig. 1A and B). These AFLs showed the typical morphological features: ductal appearance, tubular structures, and the presence of a surrounding loose stroma. Lineage tracing of adult acinar cells in $Ptf1a^{CreER}$; $LSL\text{-}Kras^{G12D}$; $R26R^{YFP}$ mice was performed that revealed YFP-positivity of AFLs and, thus, further supports the acinar origin of the lesions (Fig. 1C–F). Previously, AFLs have been localized within areas of ADM [3]. AFLs in $Ptf1a^{CreER}$; $LSL\text{-}Kras^{G12D}$ mice were also associated with ADM (Fig. 1A, C) and the amount of ADM correlated with the amount of AFLs in the pancreas (Fig. 1G).

Characterization of acinar derived atypical flat lesions

Further characterization of the acinar derived AFLs in $Ptf1a^{CreER}$; $LSL\text{-}Kras^{G12D}$ mice revealed expression of cytokeratin 19 but not carboxypeptidase A1 (CPA1), in agreement with the ductal phenotype of these lesions (Fig. 2A and B). Supporting the role as a PDAC

precursor, cytological atypia was observed in cells forming AFLs (Fig. 2C). Furthermore, proliferation as shown by frequent expression of Ki-67 was present in AFLs in *Ptf1a*^{CreER};*LSL-Kras*^{G12D} mice (Fig. 2D). In addition, P53 and pMAPK were broadly expressed in the nuclei of AFL cells (Fig. 2E and F).

Discussion

It is well established by pathological progression models that PDAC can arise from IPMN and PanIN lesions. While the former is thought to derive from ductal cells, animal models suggest an acinar origin for PanINs [4]. The recently identified AFLs are suggested to be alternative precursors for PDAC [2]. AFLs are defined as proliferative ductal lesions in areas of ADM with a peculiar surrounding stroma. The presence within areas of ADM also suggests an acinar origin for AFLs. However, this has not yet been experimentally proven. The selective recombination of *Kras*^{G12D} in adult pancreatic acinar cells in combination with lineage tracing described here supports the origin of AFLs from pancreatic acinar cells. In line with an acinar origin, previous studies have shown that recombination of oncogenic Kras, selectively in pancreatic ductal cells, does not lead to a neoplastic transformation [4,11]. However, it is unclear whether specific combinations of oncogenic insults in ductal cells could result in the formation of AFLs. Up to now, AFLs have only been shown in adult mice with expression of *Kras*^{G12D} during pancreatic embryogenesis and in families with a strong family history of PDAC [3]. To date, it was also unclear whether oncogenic insults in adult pancreatic cells can result in AFLs. Our data shows for the first time that expression of *Kras*^{G12D} exclusively in the adult pancreas is sufficient to induce AFLs.

While the clinical significance of AFLs remains to be elucidated, there is an increasing body of evidence supporting the notion that acinar cells serve as the cellular source for PDAC. Acinar cells have a remarkable plasticity in the context of oncogenic insults as they undergo ductal dedifferentiation and can form ADM, AFLs, and PanINs, all of which are presumed PDAC precursors [2]. To date, it is unclear whether the latter two develop from ADM or if these are independent routes of acinar cells on the way to become PDAC. Future studies need to show to what extent different precursors contribute to pancreatic carcinogenesis and if it is of relevance with regards to human tumor biology and therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Annett Dannemann for expert technical assistance. We thank the Institute of Pathology at the Technical University of Munich, where whole pancreatic cross-section scanning was performed.

Funding

This work was supported by grants of the Deutsche Forschungsgemeinschaft to GVF (Emmy Noether Program, FI1719/2-1) and IE (ES 285/6-I). Work in MH's laboratory was supported by funding from the National Institutes of Health (NIH CA172045).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.org/10.1016/j.pan.2017.04.014.

References

1. Ying H, Dey P, Yao W, Kimmelman AC, Draetta GF, Maitra A, et al. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev. 2016; 30(4):355–85. [PubMed: 26883357]
2. Basturk O, Hong SM, Wood LD, Adsay NV, Albores-Saavedra J, Biankin AV, et al. A revised classification system and recommendations from the baltimore consensus meeting for neoplastic precursor lesions in the pancreas. Am J Surg Pathol. 2015; 39(12):1730–41. [PubMed: 26559377]
3. Aichler M, Seiler C, Tost M, Siveke J, Mazur PK, Da Silva-Buttkus P, et al. Origin of pancreatic ductal adenocarcinoma from atypical flat lesions: a comparative study in transgenic mice and human tissues. J Pathol. 2012; 226(5):723–34. [PubMed: 21984419]
4. Kopp JL, von Figura G, Mayes E, Liu FF, Dubois CL, Morris JPT, et al. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. Cancer Cell. 2012; 22(6):737–50. [PubMed: 23201164]
5. von Figura G, Fukuda A, Roy N, Liku ME, Morris JP IV, Kim GE, et al. The chromatin regulator Brg1 suppresses formation of intraductal papillary mucinous neoplasm and pancreatic ductal adenocarcinoma. Nat Cell Biol. 2014; 16(3):255–67. [PubMed: 24561622]
6. Heiser PW, Cano DA, Landsman L, Kim GE, Kench JG, Klimstra DS, et al. Stabilization of beta-catenin induces pancreas tumor formation. Gastroenterology. 2008; 135(4):1288–300. [PubMed: 18725219]
7. Pan FC, Bankaitis ED, Boyer D, Xu X, Van de Casteele M, Magnuson MA, et al. Spatiotemporal patterns of multipotentiality in Ptf1a-expressing cells during pancreas organogenesis and injury-induced facultative restoration. Development. 2013; 140(4):751–64. [PubMed: 23325761]
8. Tuveson DA, Shaw AT, Willis NA, Silver DP, Jackson EL, Chang S, et al. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. Cancer Cell. 2004; 5(4):375–87. [PubMed: 15093544]
9. Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, et al. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol. 2001; 1:4. [PubMed: 11299042]
10. von Figura G, Morris JPT, Wright CV, Hebrok M. Nr5a2 maintains acinar cell differentiation and constrains oncogenic Kras-mediated pancreatic neoplastic initiation. Gut. 2014; 63(4):656–64. [PubMed: 23645620]
11. Bailey JM, Hendley AM, Lafaro KJ, Pruski MA, Jones NC, Alsina J, et al. p53 mutations cooperate with oncogenic Kras to promote adenocarcinoma from pancreatic ductal cells. Oncogene. 2016; 35(32):4282–8. [PubMed: 26592447]

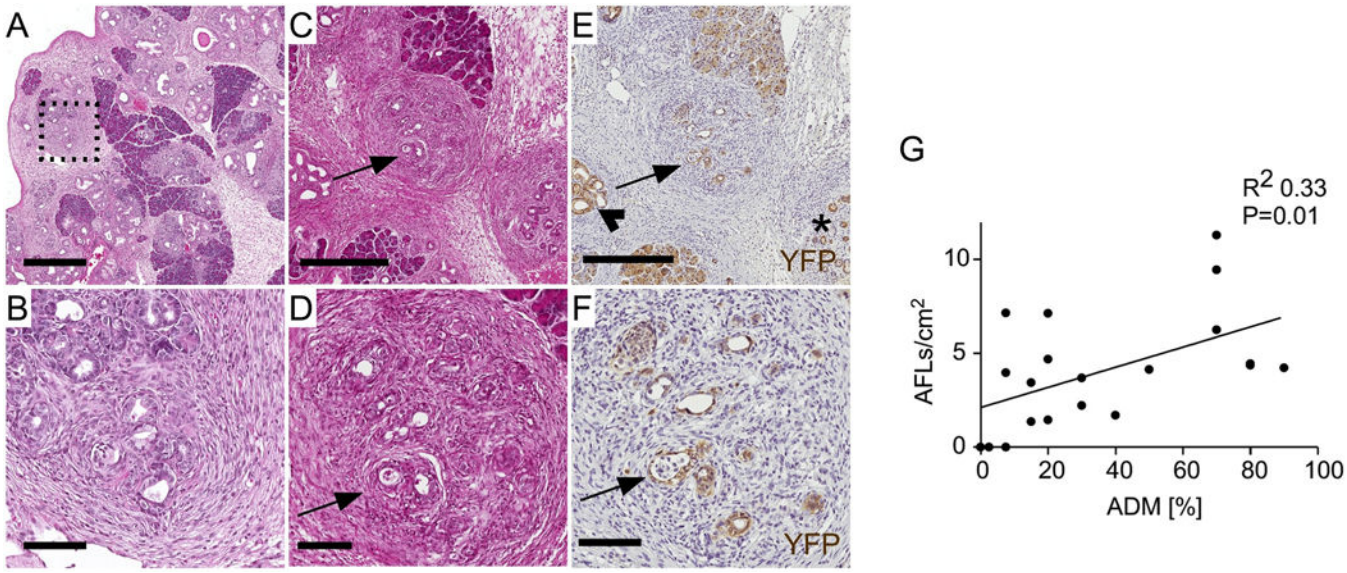

Fig. 1. Atypical flat lesions arise from pancreatic acinar cells

(A) H&E overview: AFL lesions (highlighted square) are identified in areas of ADM of *Ptf1a*^{CreER}*; LSL- Kras*^{G12D} mice. (B) Higher magnification of the AFL highlighted in (A). (C) H&E staining of an AFL lesion (arrow) in *Ptf1a*^{CreER}*; LSL-Kras*^{G12D}*; R26R*^{YFP} mice, higher magnification in (D); (E) YFP staining of AFLs from *Ptf1a*^{CreER}*; LSL-Kras*^{G12D}*; R26R*^{YFP} mice showing YFP-positivity of AFL lesions (arrow), higher magnification in (F). Note that ADM (*) and PanINs (arrowhead) are also YFP-positive. (G) Correlation of the number of AFLs with the ADM area per whole pancreatic cross-sections in Ptf1a^{CreER}; LSL- Kras^{G12D} and *Ptf1a*^{CreER}*; LSL-Kras*^{G12D}*; R26R*^{YFP} mice. Mice were analyzed at 5 months (A–F) and at 6 weeks and 4–5 months (G) after tamoxifen administration. Scale bars 500 μM (A), 400 μM (C, E), and 100 μM (B, D, F).

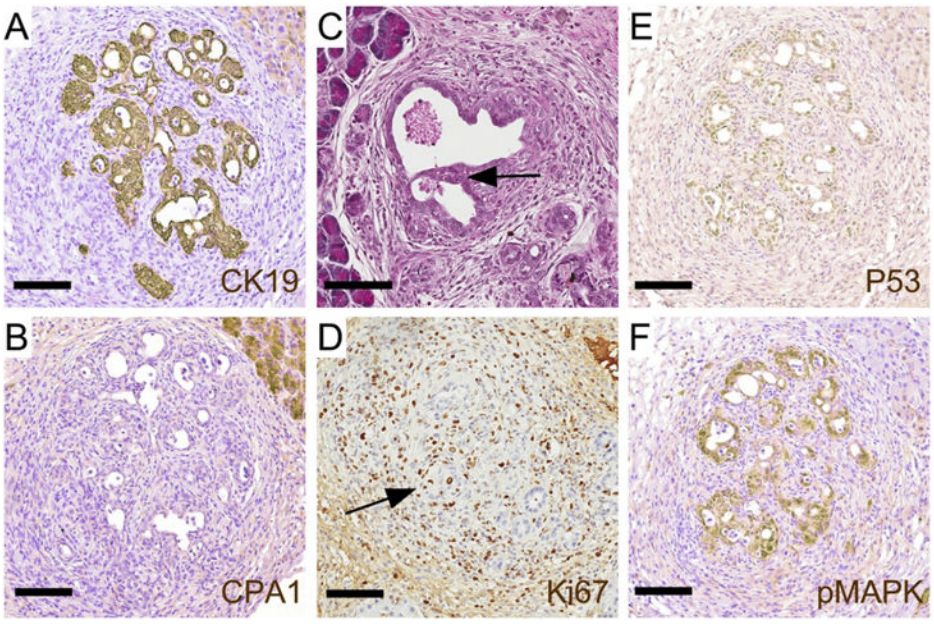

Fig. 2. Characterization of acinar derived atypical flat lesions

(A–F) Characterization of acinar derived AFLs in *Ptf1a*^{CreER}*; LSL-Kras*^{G12D} and *Ptf1a*^{CreER}*; LSL-Kras*^{G12D}*; R26R*^{YFP} mice. (A) CK19 staining reveals broad expression of the ductal marker in AFLs. (B) The acinar marker CPA1 is not expressed in AFLs. (C) Evidence of cellular atypia in acinar derived AFLs (arrow, H&E staining). (D) AFLs have a proliferative character as evidenced by an increased expression of Ki67 in the nuclei (arrow). P53 (E) and pMAPK (F) are broadly expressed in nuclei of AFLs. (A–F) Scale bars represent 100 μM; mice were analyzed at 5 months after tamoxifen administration.