© 2016. Published by The Company of Biologists Ltd | Development (2016) 143, 181 doi:10.1242/dev.133934

CORRECTION

Bicaudal C1 promotes pancreatic NEUROG3+ endocrine progenitor differentiation and ductal morphogenesis

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There were errors published in Development **142**, 858-870.

In the supplementary information, the sequences of four of the primers shown in Table S2 were incorrect. The correct sequences of these primers are as follows (5′-3′):

Hes1 mRNA reverse primer, gcgcggtatttccccaaca;

Onecut1 mRNA forward primer, agttccagcgcatgtcgg;

Onecut1 mRNA reverse primer, tttttgggggtgttgcctct;

Tcf7 mRNA reverse primer, agttcatagtacttggcctgctcttc.

The authors apologise to readers for these mistakes.

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Fig. 4. Endocrine cell decrease is caused by endocrine progenitor generation defect. (A-C) TUNEL assay (orange) followed by immunofluorescence staining for INS (cyan) and aPKC, an apical marker (magenta), on E18.5 WT and *Bicc1* KO pancreatic sections shows no apoptotic death in INS$^+$ beta cells. By contrast, TUNEL$^+$ nuclei are seen in the duct delimited by aPKC in *Bicc1* KO pancreas. E11.5 paw is used as a positive control for the TUNEL assay. (D) Percentage of MKI67$^+$INS$^+$ beta cell number among beta cells quantified on immunostained sections shows no difference between E18.5 WT and *Bicc1* KO pancreata (P=0.95). (E-G) Immunofluorescence staining for active caspase3 (CASP3) (orange) and NEUROG3 (magenta) was performed on E14.5 WT and *Bicc1* KO pancreatic sections. Insets show a magnified view of the dashed area. No apoptosis is observed in endocrine progenitor cells. E13.5 paw is used as a positive control for active CASP3. (H) Quantification of the number of pancreatic progenitors immunoreactive for PDX1 on E14.5 pancreatic sections shows no difference between WT and *Bicc1* KO pancreata (P=0.95). (I-Q) An RFP reporter under *Neurog3* promoter, Neurog3-RFP, was used to characterize the differentiation flux from NEUROG3$^+$ endocrine progenitors toward hormone$^+$ endocrine cells. Cells are first NEUROG3$^+$ (green) before becoming double positive for NEUROG3 and RFP (red). They switch off NEUROG3 and thereafter start to express hormones, INS or GCG (cyan), before switching off RFP, as exemplified in the immunofluorescence staining on sections at E14.5 in WT and *Bicc1* KO pancreas. (I) Quantification of the different cell populations on sections reveals a global 34% decrease of the NEUROG3$^+$ endocrine progenitors in *Bicc1* KO pancreata (P=0.0043), corresponding to the decrease observed in both its RFP and RFP$^+$ fractions (NEUROG3$^+$RFP , P=0.029; NEUROG3$^+$RFP$^+$, P=0.0021). The two last populations, NEUROG3 Hormone RFP$^+$ cells and Neurog3 Hormone$^+$RFP$^+$ cells, have a tendency to decrease that do not reach significance (Neurog3 Hormones RFP$^+$ cells, P=0.08; Neurog3 Hormones$^+$RFP$^+$ cells, P=0.16). Sections are counterstained with DAPI (white). For all experiments shown, n=4 for both genotypes. Scale bars: 25 µm in A-C,E,F and J-Q; 100 µm in G; 10 µm in insets. See supplementary material Table S1 for further data.

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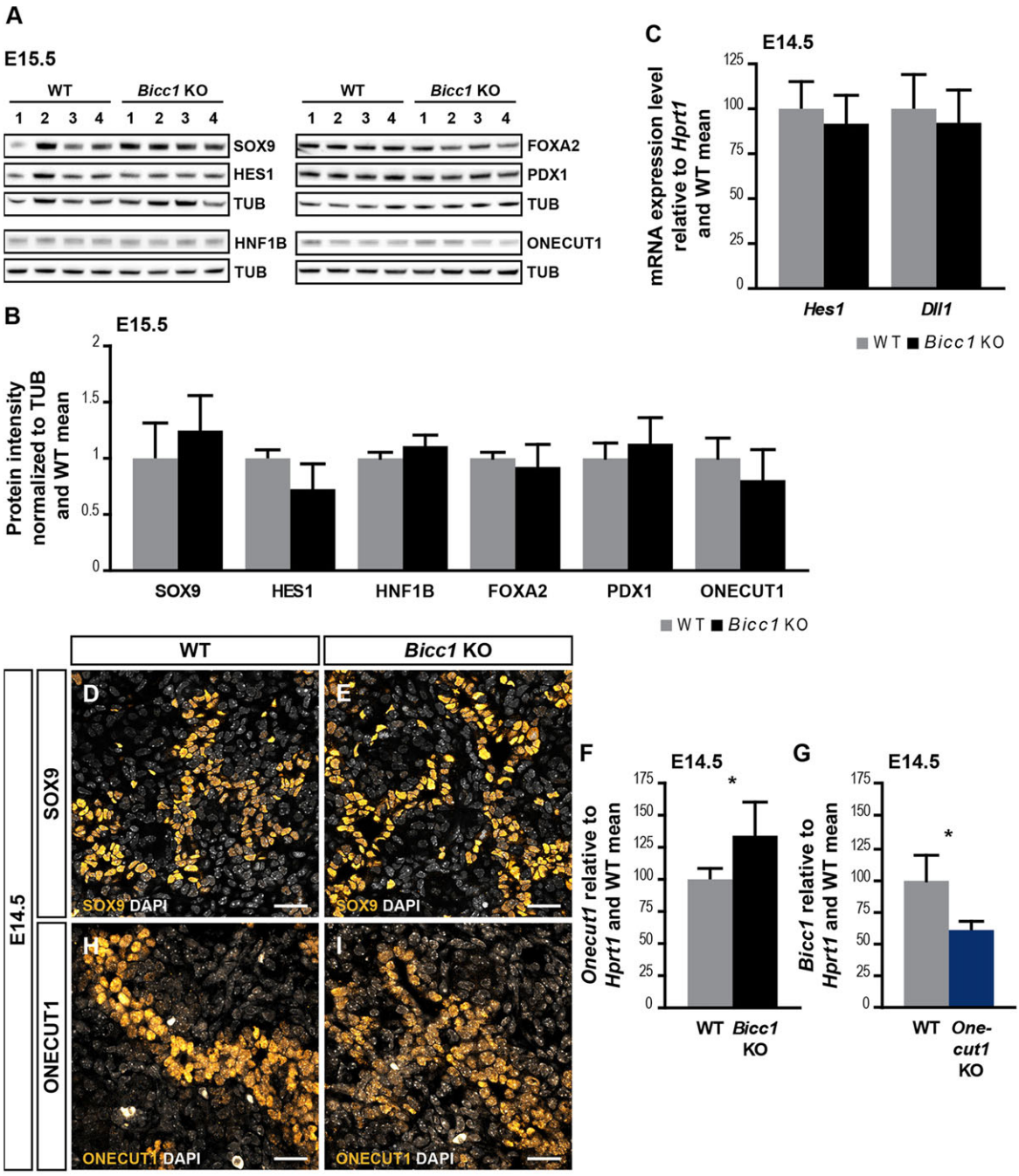

Fig. 5. BICC1 does not regulate the NOTCH pathway or known *Neurog3* transcriptional activators and functions downstream of ONECUT1. (A) Western blot performed for SOX9, HES1, HNF1B, FOXA2, PDX1 and ONECUT1 on E15.5 WT and *Bicc1* KO pancreata, and α-tubulin (TUB) as a loading control. (B) Quantification of the western blot shown in A is performed by normalizing band intensity to TUB intensity. Results are relative to WT mean. None of them are affected by *Bicc1* deletion (SOX9: WT, *n*=4; *Bicc1* KO, *n*=4, *P*=0.30; HES1: WT, *n*=4; *Bicc1* KO, *n*=4, *P*=0.06; HNF1B: WT, *n*=4; *Bicc1* KO, *n*=4, *P*=0.10; FOXA2: WT, *n*=4; *Bicc1* KO, *n*=4, *P*=0.48; PDX1: WT, *n*=4; *Bicc1* KO, *n*=4, *P*=0.37; ONECUT1: WT, *n*=7; *Bicc1* KO, *n*=8, *P*=0.13). (C) qPCR analysis for *Hes1* and *Dll1* performed on E14.5 WT and *Bicc1* KO dorsal pancreata does not show any differences (*Hes1*: WT, *n*=4; *Bicc1* KO, *n*=5, *P*=0.45; *Dll1*: WT, *n*=4; *Bicc1* KO, *n*=5, *P*=0.55). (D,E) Immunofluorescence staining performed on E14.5 WT (*n*=4) and *Bicc*1 KO (*n*=4) pancreatic sections do not show SOX9 (orange) expression pattern differences between both genotypes. (F) qPCR analysis of *Onecut1* in WT versus *Bicc1* KO E14.5 dorsal buds reveals a 34% increase in *Onecut1* transcript in the KO (WT, *n*=4; *Bicc1* KO, *n*=5; *P*=0.042). (G) qPCR analysis of *Bicc1* in WT versus *Onecut1* KO E14.5 dorsal buds shows a decrease by 40% of *Bicc1* transcript in the *Onecut1* KO (WT, *n*=4; *Onecut1*, *n*=3; *P*=0.027). (H,I) Immunofluorescence staining for ONECUT1 on E14.5 WT (*n*=2) and *Bicc1* KO (*n*=2) pancreatic sections shows no expression pattern difference. qPCR results are normalized to the housekeeping gene *Hprt1*. Results are represented in percentage of WT mean. Sections are counterstained with DAPI (white). Scale bars: 25 µm. See supplementary material Table S1 for further data.

(Fig. 5A,B). Although *Onecut1* mRNA was increased by 34% in *Bicc1* KO compared with WT pancreas (Fig. 5F), its protein level was not changed (Fig. 5A,B), and the localization of the protein was normal (Fig. 5H,I). In conclusion, NEUROG3^{+} cell reduction is not due to a decrease of its activators SOX9, FOXA2, PDX1, HNF1B and ONECUT1 or to NOTCH pathway alteration.

Onecut1 KO embryos also develop pancreatic cysts combined with a decrease in endocrine cells due to defective endocrine

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progenitor differentiation (Lynn et al., 2007). As the experiments above showed that BICC1 does not promote ONECUT1 expression, we investigated whether BICC1 functions downstream of *Onecut1*. Indeed, we found that *Bicc1* mRNA was decreased by 40% in *Onecut1* KO pancreata (Fig. 5G), suggesting that ONECUT1 promotes *Bicc1* expression.

Although BICC1 was previously shown to control Wnt signaling in the node and in a cell line reporter assay (Maisonneuve et al., 2009), we did not detect any change in *Axin2* and *Tcf7*, two Wnt targets, nor any alterations in the expression of the *Axin2-lacZ* reporter in the pancreata of *Bicc1* KOs at E14.5 (supplementary material Fig. S5).

Cyst formation is associated with PKD2 downregulation and immune cell infiltration

To decipher the molecular mechanisms underlying cyst formation in *Bicc1* KO pancreata, E13.5 *Bicc1* KO and WT pancreas transcriptomes were compared by RNA sequencing. At this stage, duct enlargement was not yet observed, allowing us to detect expression changes prior to cyst formation rather than as a consequence of it. Only few genes were significantly upregulated or downregulated with an FDR<0.05 (Tables 1 and 2). All upregulated genes were expressed at low levels, with an rpkm<1 in WT pancreata. Among the downregulated genes, *Pkd2* was decreased 1.9-fold in *Bicc1* KO compared with WT pancreata. PKD2 deficiency results in pancreas and kidney cyst formation (Wu et al., 2000; Chang et al., 2006). PKD2 was expressed in the pancreatic ducts and appeared more weakly expressed in *Bicc1* KO. Moreover, western blot revealed a 2.1-fold decrease in PKD2 protein levels in *Bicc1* KO pancreata at E15.5, suggesting that regulation of PKD2 by BICC1 operates before translation (Fig. 6A-D).

Due to the small number of differentially regulated genes, it was not possible to perform a gene ontology analysis. Nevertheless, an important proportion of both up- and downregulated genes were related to the immune system, such as *S100a8*, *S100a9*, *Crp* and *Cma1*, thereby arguing for an immune status change (Tables 1 and 2). To further investigate this observation, the number of cells stained for CD45, a pan-immune cell marker, was quantified in *Bicc1* KO and WT E14.5 pancreata. It revealed a twofold increase in the *Bicc1* KO (Fig. 6E-G), with their presence correlated with the extent of cysts. Many of these cells were macrophages expressing EMR1, also called F4/80 (Fig. 6H-K). Moreover, tissue surrounding the cysts at E18.5 also exhibited numerous $CD45^+$ cells and $EMR1^+$ cells. The cysts at this stage were also surrounded by smooth muscle $actin^+$ ($ACTA2^+$) cells, suggestive of fibroblast activation (Apte et al., 1999; Haber et al., 1999) (Fig. 6L-O). $ACTA2^+$ cells were only present at E14.5 in a subset of *Bicc1* KO pancreata (Fig. 6H-K), which suggests a secondary effect of cyst formation and/or of immune cell recruitment. In conclusion, cysts arose in a context of PKD2 decrease and immune cell infiltration with stromal reaction.

Table 1. Upregulated mRNA in E13.5 *Bicc1* KO pancreas

Gene symbol	WT mean (rpkm)	*Bicc1* KO mean (rpkm)	Fold change	FDR
Slc16a3	0.89	2.02	2.27	0.0049
Gm15745	0.16	6.51	41.82	0.0006
RP23-281E24.2	0.49	1.87	3.85	<0.0001
Cma1	0.38	2.23	5.84	0.0009
Six2	0.18	2.67	15.06	0.0004
Fam162b	0.22	1.05	4.77	<0.0001
Hdc	0.10	0.32	3.12	0.0446
Rpl30-ps5	0.09	0.28	3.24	0.0473
Myod1	0.09	0.67	7.14	<0.0001
Nkx2-5	0.06	0.46	7.83	0.0321
Mcpt4	0.01	0.22	16.23	<0.0001
1700023F06Rik	0.01	0.50	50.43	<0.0001

mRNAs found upregulated in *Bicc1* KO compared with WT pancreas are ordered according to NIA array analysis tool. The ranking is based on a combination of their expression level in read per kilobase per million (rpkm), their fold change and their FDR.

Table 2. Downregulated mRNA in E13.5 *Bicc1* KO pancreas

Gene symbol	WT mean (rpkm)	*Bicc1* KO mean (rpkm)	Fold change	FDR
Bicc1	19.12	4.43	4.31	<0.0001
S100a9	11.63	2.10	5.54	0.0083
Pah	16.42	4.78	3.43	0.0118
S100a8	7.10	1.26	5.62	0.0111
Calcr	16.85	8.74	1.93	<0.0001
2010107G23Rik	30.82	20.19	1.53	0.0321
Pkd2	10.57	5.63	1.88	0.0001
Crp	23.85	15.71	1.52	0.0092
4930533K18Rik	4.95	1.48	3.34	<0.0001
BC100530	3.07	0.50	6.10	0.0118
Slc5a9	2.30	1.22	1.88	0.0481
Cer1	2.19	1.03	2.13	0.009
Ngp	2.69	0.39	6.88	0.0027
Anxa9	1.13	0.41	2.77	0.0006
Gm5483	1.31	0.23	5.77	0.0004
Gm13305	0.84	0.23	3.66	<0.0001
Olfm4	1.48	0.19	7.83	0.0001
Il11ra2	0.41	0.06	7.22	<0.0001
Gm2002	0.49	0.02	26.95	<0.0001

mRNAs found downregulated in *Bicc1* compared with WT pancreas are ordered according to NIA array analysis tool. The ranking is based on a combination of their expression level in rpkm, their fold change and their FDR.

DISCUSSION

BICC1 integrates epithelial morphogenesis and differentiation

We show here that BICC1 integrates appropriate pancreatic ductal tree morphogenesis and differentiation of endocrine cells originating from the progenitor-lined ducts. The absence of BICC1 causes cysts in both pancreatic and liver ducts, in addition to kidney dysplasia (Maisonneuve et al., 2009; Tran et al., 2010). Moreover, we show that BICC1 normally potentiates $NEUROG3^+$ endocrine progenitor production, leading to endocrine cell reduction in *Bicc1* KO pancreata (Fig. 7). These defects occur relatively late in pancreas development: BICC1 expression is elevated at E12.5 in bipotent ducto-endocrine progenitors, and upon the loss of *Bicc1*, NEUROG3 is decreased at E14.5 (RNA and cell number) but not yet at E13.5 (RNA sequencing). Endocrine cell decrease follows, being detected at E18.5 but not yet at E14.5. Our long-term lineage tracing shows a sustained decrease of 30-50% of endocrine cell production from E14.5 onwards. Glucagon cell production is therefore less affected in the *Bicc1* KO, as they are produced in large numbers before E14.5 (Johansson et al., 2007). $Neurog3^+$ progeny were globally reduced to the same extent as endocrine cells, indicating that BICC1 does not control endocrine cell production downstream of *Neurog3*.

BICC1 targets mediating endocrine cell differentiation

BICC1 is either able to repress translation by facilitating miRNA binding (Piazzon et al., 2012) or to enhance translation by inhibiting miRNA activity, thus stabilizing protein levels of the targets more than RNA levels (Tran et al., 2010; Gamberi and Lasko, 2012). We ruled out that BICC1 directly regulates *Neurog3*

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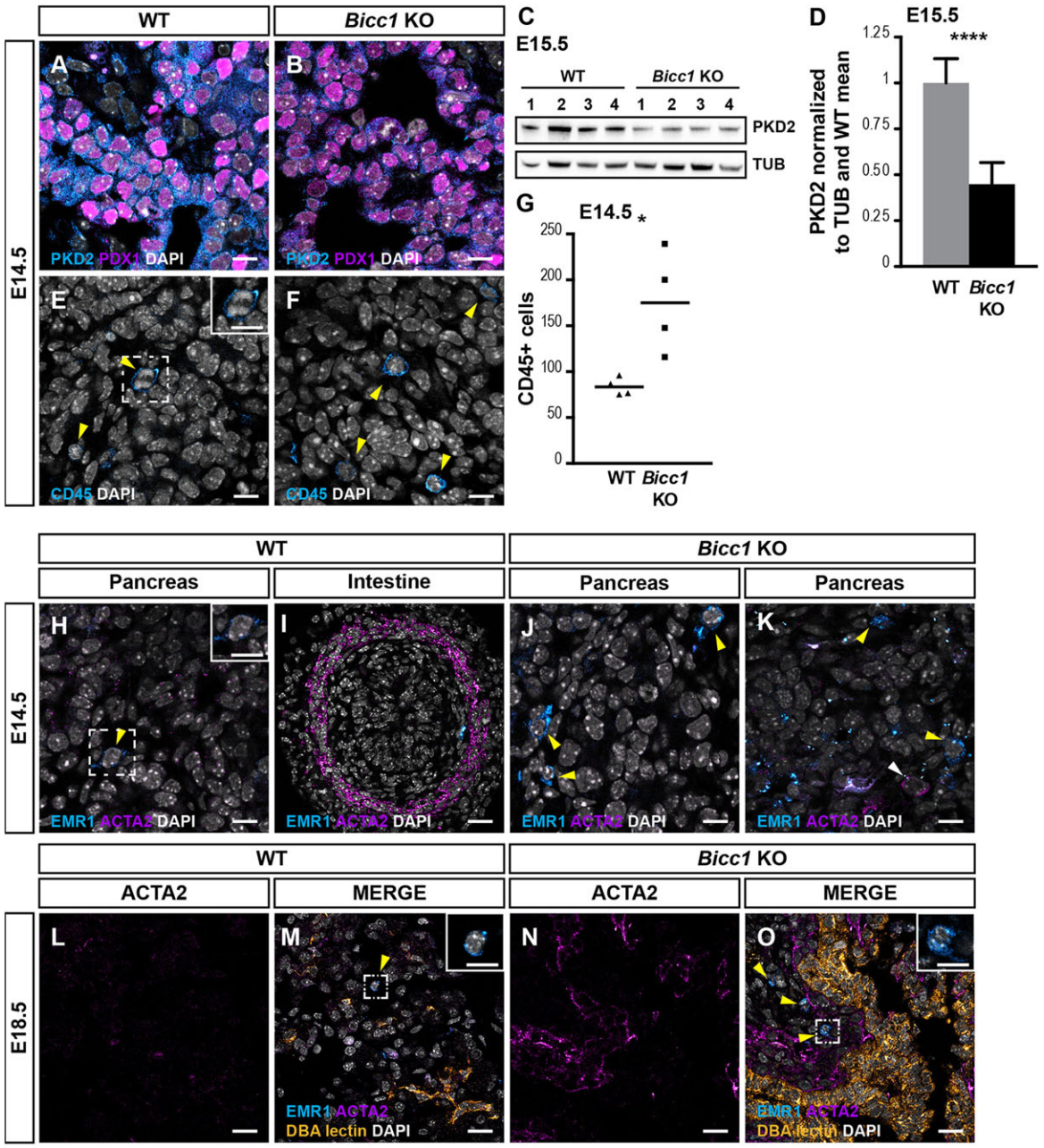

Fig. 6. PKD2 reduction and immune cell infiltration in *Bicc1* KO pancreas. (A,B) E14.5 WT and *Bicc1* KO pancreatic sections were stained by immunofluorescence for PKD2 (cyan) and PDX1 (magenta), highlighting pancreatic epithelium. E14.5 *Bicc1* KO pancreata (n=4) have a marked PKD2 decrease in the epithelium compared with WT littermate pancreata (n=4). (C) Western blot for PKD2 in E15.5 WT and *Bicc1* KO pancreata and TUB as a loading control. (D) Quantification of the western blot shown in C. PKD2 intensity is normalized to TUB intensity, and results are relative to WT mean. PKD2 in *Bicc1* KO pancreas is decreased 2.1-fold (WT, n=8; *Bicc1* KO, n=8; P<0.0001). (E,F) Immunofluorescence staining for CD45 (cyan), a pan-immune marker (yellow arrowheads), is performed on E14.5 WT and *Bicc1* KO pancreatic sections. Inset in E shows a high magnification of the dashed area. (G) Quantification of the immunostaining shows a 2.1-fold increase of $CD45^+$ cells in *Bicc1* KO (WT, n=4; *Bicc1* KO, n=4; P=0.017). (H-K) Immunofluorescence staining performed on E14.5 WT (n=5) and *Bicc1* KO (n=6) pancreata for EMR1 (cyan), a macrophage marker, and ACTA2 (magenta). E14.5 WT intestinal wall is used as a positive control for ACTA2. Both genotypes show $EMR1^+$ macrophages in the pancreas. Whereas $ACTA2^+$ cells are not observed in E14.5 WT pancreata, they were present in a subset of *Bicc1* KO pancreata. (L-O) Immunofluorescence staining for ACTA2 (magenta), EMR1 (cyan) and DBA lectin (orange) performed on E18.5 WT and *Bicc1* KO pancreatic sections. $ACTA2^+$ cells surround cysts (n=4), but not WT ducts (n=4). Macrophages (yellow arrowheads) are present around cystic ducts in E18.5 *Bicc1* KO pancreata (n=2), whereas there are only a few, scattered macrophages in WT pancreata (n=4). Insets show high magnification views of the dashed boxes. Sections are counterstained with DAPI (white). Scale bars: 10 µm in A,B,E,F and insets; 20 µm in H-O. See supplementary material Table S1 for further data.

stability or translation, as the reduction in number of cells expressing transcriptional reporters (Neurog3-Cre and Neurog3-RFP) is similar to the reduction in number of pro-endocrine cells expressing NEUROG3 protein. BICC1 might thus either enhance a transcriptional activator of *Neurog3* or inhibit a transcriptional repressor. HES1, a transcriptional repressor of *Neurog3*, was not changed in *Bicc1* KO pancreas, and neither were SOX9, FOXA2, PDX1 and HNF1B, some of its transcriptional activators (Lee et al.,

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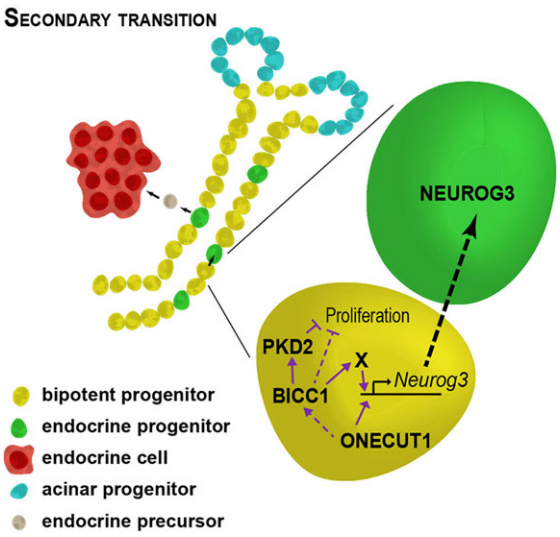

Fig. 7. Proposed model of BICC1 function during pancreas development. During the secondary transition, bipotent ductal progenitors (yellow) can differentiate into NEUROG3$^+$ endocrine progenitors (green). These progenitors leave the ducts (gray) while differentiating into endocrine cells (red). After the onset of the secondary transition, BICC1 is present in bipotent ductal progenitors (yellow). It regulates PKD2 and thereby inhibits ductal over-proliferation maintaining ductal homeostasis. BICC1 might also inhibit proliferation via other targets. It also promotes *Neurog3* expression via an unknown factor, enabling the differentiation toward endocrine progenitor (green). Whereas ONECUT1 directly activates *Neurog3* (Jacquemin et al., 2000), it also promotes *Bicc1* expression.

2001; Shih et al., 2012; Ejarque et al., 2013). ONECUT1 also activates *Neurog3* expression (Jacquemin et al., 2000), but although *Onecut1* was upregulated at the mRNA level, its protein level was unchanged, arguing against it being targeted by BICC1 (Fig. 7). Other transcription factors activate the *Neurog3* promoter, such as ONECUT2, HNF1A, GLIS3 and MYT1 (Lee et al., 2001; Vanhorenbeeck et al., 2007; Wang et al., 2007, 2008; Yang et al., 2011). It would thus be interesting to see whether these putative targets are affected by *Bicc1* deletion. The role of BICC1 in targeting mRNAs by miRNAs implies that its action is not seen at the mRNA level, as many miRNAs do not regulate transcript levels. It might explain why none of the genes above were changed in the transcriptome analysis of *Bicc1* KO versus WT pancreata. Few BICC1 targets are currently known, and the identification of its binding mRNA partners would give better insight into the generation of NEUROG3$^+$ endocrine progenitors.

BICC1 controls epithelial morphogenesis downstream of ONECUT1 and upstream of PKD2

Bicc1 KO pancreatic phenotype partially recapitulates the *Onecut1* KO phenotype. Indeed, in both cases, there are ductal cysts and fewer endocrine cells, due to a failure to produce endocrine progenitors (Jacquemin et al., 2000). We show that ONECUT1 controls *Bicc1* transcript levels. ONECUT1 might activate *Bicc1* expression directly, but an indirect control via HNF1B is plausible. Indeed, ONECUT1 enhances *Hnf1b* expression, and the latter is able to induce *Bicc1* expression in the kidney (Maestro et al., 2003; Verdeguer et al., 2010).

Cyst formation is a shared feature of several mutants affecting pancreas development. Cilia mutants have cysts in the pancreas, and *Onecut1* KOs lack cilia at early stages (Cano et al., 2004, 2006; Pierreux et al., 2006). However, the presence of cilia has been reported in the cochlea and node of *Bicc1* KOs (Maisonneuve et al., 2009; Piazzon et al., 2012), and we also observed them in their pancreata. However, BICC1 affects cilia position on the cells and their ability to synergistically rotate in the node. These cilia normally generate a flow of extracellular signals creating left-right asymmetry in embryos. In *Bicc1* KOs, the fluid flow randomization thus leads to left-right randomization (Maisonneuve et al., 2009). Their position and function is difficult to assess in the tortuous pancreatic ducts but is possibly altered.

Other mouse mutants also exhibit pancreatic cysts. *Neurog3* KOs harbor cysts smaller than those seen in *Bicc1* KOs (Magenheim et al., 2011). However, these cysts are thought to originate from an accumulation of endocrine progenitors failing to further differentiate, which is not the case in *Bicc1* KOs. It is also unlikely that the presence of cysts causes the endocrine defect, as certain gene KOs have normal endocrine cell numbers in spite of the presence of cysts (Cano et al., 2004, 2006). *Sox9* KO pancreata also have cysts due to an expanded ductal population, but SOX9 is not affected in *Bicc1* KOs. SOX9 and BICC1 appear to prevent cyst formation independently of each other by controlling *Pkd2* expression (Shih et al., 2012). *Pkd2* KO embryos and heterozygotes acquiring a spontaneous mutation of the second allele exhibit cysts in the pancreas and in the kidneys (Wu et al., 2000; Chang et al., 2006). In the kidneys, BICC1 inhibits targeting of *Pkd2* by miR-17, thus stabilizing PKD2 (Tran et al., 2010). PKD2 also functions downstream of BICC1 in osteoblasts, and knockdown of *Bicc1* is rescued by overexpressing *Pkd2* (Mesner et al., 2014). Together, these findings suggest that PKD2 is a major mediator of BICC1 activity in multiple organs.

An indirect and early mesenchymal contribution to cyst formation

A conserved feature of cyst formation in kidney is its association with fibrosis and infiltrating macrophages. Activated macrophages have been shown to promote cyst formation in polycystic kidney disease models (Karihaloo et al., 2011; Swenson-Fields et al., 2013; Ta et al., 2013). However, macrophage infiltration is insufficient to induce cyst formation in the fetal pancreas (Geutskens et al., 2005). In adults, however, macrophages are able to activate pancreatic stellate cells, the fibroblasts present in the pancreas (Schmid-Kotsas et al., 1999). Once activated, stellate cells start to express ACTA2 and promote fibrogenesis by producing extracellular matrix component (Masamune et al., 2009; Shi et al., 2014). In *Bicc1* KOs, the expansion of resident or infiltrating macrophages that we uncovered might promote cyst formation and at the same time activate stellate cells. Indeed, whereas immune cells were present in all pancreata as early as E14.5, ACTA2$^+$ cells were present in some *Bicc1* KO pancreata and became more prevalent at E18.5.

A role for BICC1 in syndromes associating kidney defects and diabetes?

Heterozygous *BICC1* mutations in human have been identified and associated with kidney dysplasia (Kraus et al., 2012). Other syndromes associate kidney defects with diabetes and/or pancreatic dysplasia. Indeed, MODY5 syndrome, in which *HNF1B* is mutated, is characterized by kidney cysts and diabetes (Bellanné-Chantelot et al., 2005). Both HNF1B and BICC1 prevent kidney cysts and control pancreas development, which might be explained by the ability of HFN1B to activate *Bicc1* directly (Haumaitre et al., 2006; Verdeguer et al., 2010) or indirectly (De Vas et al., 2015). It will be important to investigate whether *BICC1* mutations contribute to syndromes associated with kidney cysts and either diabetes or pancreatic

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dysplasia or to link *BICC1* with already identified genes in such syndromes. Other monogenic forms of type 2 diabetes might also be associated with kidney dysplasia. For example, *GLIS3* mutations can cause neonatal diabetes associated with cystic kidneys (Senée et al., 2006). We have also observed a stromal reaction in *Bicc1* KOs, which leads us to think that some patients with Ivemark syndrome, who exhibit cystic kidneys and pancreatic fibrosis, might bear *Bicc1* mutations (Vankalakunti et al., 2007). The genetic causes of this familial syndrome have been identified in only a subset of cases, and include mutations in nephronophthisis 3 (*NPHP3*) (Bergmann et al., 2008; Fiskerstrand et al., 2010). These studies will be important for genetic counseling and patient management.

MATERIALS AND METHODS

Mice and genotyping

Mus musculus were either housed at EPFL, Switzerland, at the University of Copenhagen, Denmark, or at UCL, Belgium. The Service de la consommation et des affaires vétérinaires, Vaud in Switzerland, the Commission d'Ethique d'Expérimentation Animale of UCL in Belgium or the Dyreforsøgstilsynet in Denmark approved the mouse housing and experiments in their respective countries. Bicc1tm1Bdc (Bicc1) (Maisonneuve et al., 2009), Tg(Ngn3-tRFP)AGB (*Neurog3-RFP*) (Kim et al., unpublished data), Gt(ROSA)26Sortm1(EYFP)Cos (*Rosa YFP*) (Srinivas et al., 2001), Tg(Neurog3-cre)C1Able Ngn3-Cre (*Neurog3-Cre*) (Schonhoff et al., 2004) and *Onecut1* (Jacquemin et al., 2000) B6.129P2-Axin2tm1Wbm/J (*Axin2-lacZ*) (Lustig et al., 2002) mouse lines were used for this study. Genotyping primers and conditions are listed in supplementary material Table S2.

Specimen preparation

Guts, pancreas or dorsal pancreatic buds were dissected from mouse embryos at different stages or postnatally and treated as described by Cortijo et al. (2012). Cryostat sections (7 µm thick) were collected. For quantification, systematic uniform random-sampled sections (SUR sections) were collected every 5, 6 and 10 sections for E12.5, E14.5 and E18.5/P0, respectively.

Histology, immunofluorescence, X-Gal staining and TUNEL assay

Haematoxylin & eosin (H&E) staining was performed on cryosections. Immunofluorescence was performed as described by Cortijo et al. (2012). Antibodies and the dilution used in this study are listed in supplementary material Table S3. TUNEL assay was performed with an ApopTag fluorescein direct *in situ* apoptosis detection kit (Millipore, S7160) and followed by immunofluorescence staining as described by Cai et al. (2012). X-Gal staining was performed as described by Dessimoz et al. (2005).

Images and image analyses

A Leica SP8 confocal microscope, a Leica DM5500 upright wide-field microscope and a 3DHISTECH panoramic MIDI slide scanner were used for imaging. Quantifications were performed either manually or automatically by counting stained positive-cell profiles harboring a nucleus. See supplementary materials for an extended procedure description.

Western blot

E15.5 pancreata were lysed and sonicated in Laemmli buffer. After protein separation by electrophoresis and transfer, the membrane was incubated overnight with primary antibody. It was detected by chemiluminescence after HRP-conjugated secondary antibody incubation. Membranes were stripped and reprobed. See supplementary materials for an extended protocol. Antibodies and the dilution used in this study are listed in supplementary material Table S3. For comparison, the protein of interest was normalized to α-tubulin (TUB) level. Results are relative to WT mean.

RNA extraction and RT-qPCR

RNA was extracted from E10.5, E11.5, E12.5 and E14.5 dorsal bud using RNeasy Mini Kit (QIAGEN, 74104) including a DNase treatment with RNase-free DNase (QIAGEN, 79254) following the manufacturer instructions. 100 ng to 500 ng of RNA was then reverse-transcribed into cDNA with SuperScript III reverse transcriptase (Life Technologies, 18080-044) following the manufacturer's protocol. qPCRs were performed on 1/5 to 1/50 of the synthetized cDNA on the StepOnePlus real-time PCR system (Life Technologies) using Power SYBR Green as dye (Applied Biosystems, 4368577) and analyzed as described in Thompson et al. (2012). For comparison, the gene of interest was normalized to the housekeeping gene *Hprt1*. Results are indicated as percentage of the WT mean. Primer sequences and annealing temperatures are listed in supplementary material Table S2.

RNA sequencing

RNA was extracted from E13.5 dorsal bud using an RNeasy plus micro kit (QIAGEN, 74034) following the manufacturer protocol allowing small RNA elution. Three samples of the same genotype were then pooled together. TruSeqRNA libraries were synthetized from 500 ng of three WT and three *Bicc1* KO pools. Libraries were sequenced on an Illumina HiSeq 2000 platform (three replicates per condition, 100 nt single-end reads) and mapped to the mm9 mouse genome with bowtie v0.12.7 (480 million reads mapped in total) (Langmead et al., 2009). The data were then filtered to eliminate genes which had read per kilobase per million (rpkm) below 0.01, and were analyzed using the NIA array analysis tool (http://lgsun.grc.nia.nih.gov/ANOVA/). Differences with an FDR<0.05 were considered statistically significant. RNA sequencing data are available on NCBI Gene Expression Omnibus (accession number: GSE58833).

Statistical analysis

Statistical analyses were performed with GraphPad Prism4 and 6 software packages and Microsoft Excel. Results were indicated by the mean±s.d. except for Fig. 6G, which shows a dot plot with the mean. Differences assessed with *t*-test (assuming normal distribution) were considered statistically significant when the *P*-value was <0.05.

Acknowledgements

The authors would like to thank A. Sharov for his contribution to RNA sequencing data analysis and W. Hamilton for his comments on the manuscript. They also thank the group of J. Brickman, the group of P. Serup and the group of H. Semb for reagents.

Competing interests

The authors declare no competing or financial interests.

Author contributions

L.A.L. designed, carried out the experiments and wrote the manuscript. J.G. performed preliminary experiments analyzing the *Bicc1* KO phenotype and the OCT. Y.H.K. generated Neurog3-RFP mouse line. J.R. and S.C. analyzed the RNA sequencing data. P.J. collected *Onecut1* KO samples and their WT littermates. D.B.C. generated the Bicc1 mouse line. A.G.-B. designed the experiments and wrote the manuscript. All authors revised the manuscript.

Funding

This work has been supported by a Swiss National Fund Sinergia grant [CRSI33_130662] and a Novo Nordisk Found grant [nordic endocrinology 5099].

Supplementary material

Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.114611/-/DC1

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